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- (71) 申请人(对除美国以外的所有指定国): 深圳市清华源 兴生物医药科技有限公司(SHENZHEN TSINGHUA YUANXING BIO-PHARM SCIENCE & TECHNOLOGY CO., LTD.) [CN/CN]; 中国广东省 深圳市南山区高新技术开发区北区郎山路, Guangdong 518057 (CN)。
- (72) 发明人;及
- (75) 发明人/申请人(仅对美国): 田聆(TIAN, Ling) [CN/CN]; 中国四川省成都市小南街123号冠城花园檀香阁08-48室, Sichuan 610015 (CN)。魏于全(WEI, Yuquan) [CN/CN]; 中国四川省成都市大学路12号三小区14栋三单元6楼18号, Sichuan 610041 (CN)。
- (74) 代理人: 北京纪凯知识产权代理有限公司(JEEKAI & PARTNERS); 中国北京市西城区宣武门西大街129号金隅大厦602室, Beijing 100031 (CN)。

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— 包括国际检索报告。

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- (54) Title: METHOD OF PREPARING A VACCINE AND ANTI-TUMOR VACCINES
- (54) 发明名称: 疫苗制备方法和抗肿瘤疫苗
- (57) Abstract: The present invention provides a method of preparing a vaccine. Said method includes: 1) analyzing specific antigen of particular pathogen; 2) obtaining a polynucleotide encoding the specific antigen; 3) obtaining a polynucleotide sequence having a sufficient difference from the polynucleotide; 4) preparing a vaccine using the polynucleotide sequence. The present invention also provides anti-tumor vaccines, particularly anti-tumor EGFR molecular vaccines. Said anti-tumor EGFR molecular vaccines are a variety of new biotechnological vaccines constructed by using EGFR moleculars as anigens, including protein vaccines, gene vaccines, gene-modified vaccines and stably transformed symbiotic bacterium. One of the biological function of the EGFR molecular vaccines has an anti-tumor effect on a variety of solid tumor cancer which over-express the EGFR molecular, e.g., lung cancer, breast cancer, ovary cancer, colon cancer, prostate cancer, stomach cancer, etc, including a protective anti-tumor immune effect, therapeutic anti-tumor effect and inhibiting cancer metastasis. The anti-tumor action mechanism of EGFR molecular vaccine is that said EGFR moleculars may destroy an individual's immune tolerance to self-EGFR molecular as cross-reactive antigens, and therefore induce an immuno-cross-response to the self-EGFR molecular in the individual, including active immune reaction (cellular immune reaction and humoral immune reaction) and passive immune reaction (adoptive immune reaction). Said anti-tumor EGFR molecular vaccines are either traditional preparation (aqua, lyophilized powder) or nano preparation prepared by nano biotechnology. Said EGFR molecular vaccine exhibits an enhanced anti-tumor effect through the synergistic action of other immunopotentiator.

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(57) 摘要

本发明提供了一种制备疫苗的方法。该方法包括: 1)分析特定病原体的特异抗原; 2)获得编码特异抗原的多核苷酸序列; 3)获得与该多核苷酸序列有足够差异的多核苷酸序列; 4)利用步骤 3)中所得的多核苷酸序列制备疫苗。本发明也提供了抗肿瘤疫苗,特别是 EGFR 分子疫苗。抗肿瘤 EGFR 分子疫苗是利用经现代生物工程技术改造进化的或在生物进化过程中形成的来源于人或其他异种生物的同源EGFR 分子作为抗原而构建的各种新生物技术疫苗,包括蛋白疫苗、基因疫苗、病毒疫苗、基因修饰疫苗和稳定转化共生菌。EGFR 分子疫苗的一个重要生物学功能就是对各种过表达 EGFR 分子的实体瘤,包括肺癌、乳腺癌、卵巢癌、结肠癌、前列腺癌、胃癌等,都具有抗肿瘤作用效应,包括保护性抗肿瘤作用、治疗性抗肿瘤作用以及抗肿瘤转移作用。EGFR 分子疫苗的抗肿瘤作用机制在于:EGFR 分子作为免疫交叉抗原,能打破机体对自身 EGFR 分子的免疫耐受,诱导机体产生针对 EGFR 分子自身免疫样交叉反应,包括主动免疫反应(细胞免疫反应和体液免疫反应)和被动免疫反应(过继免疫反应)。抗肿瘤 EGFR 分子疫苗可以是常规制剂(水剂、冻干粉),也可以是利用纳米生物技术制备而成的纳米粒制剂。EGFR 分子疫苗的抗肿瘤作用还可在其它免疫促进因子的协同作用下得到增强。

疫苗制备方法和抗肿瘤疫苗

技术领域

本发明涉及生物技术领域,具体而言涉及一种疫苗制备方法和制备的生物疫苗,特别是新型的抗肿瘤疫苗,如 EGFR 分子疫苗。分子疫苗属新生物技术药物(New Biotechnological drug)范畴,涉及到多种生物技术,如 PCR 技术、分子克隆技术、基因表达技术、现代疫苗技术、以及生物技术药物学和药剂学等。

背景技术

疫苗(vaccine)是一种能刺激机体免疫系统产生抗特异性靶物质(如病毒、细菌 等)的免疫反应的物质。经典的疫苗概念来源于抗感染免疫,它主要是使用处理过 的病原微生物(如病毒、细菌等)及其衍生物免疫机体产生体液免疫反应以预防感染 性疾病。比如,灭活疫苗(inactivated vaccine)是利用化学方法杀死实际感染成分 的衍生物。弱毒疫苗(attenuated vaccine)是改变过的活病毒或细菌使其在接种的 生物体内不能增殖。这两类疫苗都是通过表面蛋白(抗原)作用于 B 和 T 淋病细 胞来进行免疫的。当病原体生物感染人体时,B和T淋病细胞就会迅速作出反应, 在病原微生物产生破坏之前将其消灭。然而,这两类疫苗潜藏着危险,因为它们 能被传染性病菌所污染。例如,每年总有一小部分儿童因为接种脊髓灰质炎疫苗 而感染此病。因此,一个发展方向是亚单位疫苗(subunit vaccine)。亚单位疫苗是 利用 DNA 重组技术生产的单纯由表面蛋白组成、能引起免疫系统反应的疫苗,也 称为基因工程疫苗或者 DNA 重组疫苗。参见,基因工程学原理,马建岗,主编, 西安交通大学出版社,p240,2001.11。除了上述采用病原体本身、其具有免疫原 性的构成成分、以及基因表达产物外,也有人研究使用"裸"DNA 作为疫苗的基因 疫苗,是由来源于病原体的一个抗原编码基因及作为其载体的质粒 DNA 组成。通 过注射或粒子轰击等途径将基因疫苗导入人体后,这段基因可以在活体细胞中合 成抗原蛋白,从而引起机体免疫反应。目前,基因表达文库免疫技术是发现免疫 活性基因的最系统和客观的手段。参见, 第四次浪潮: 生物经济, 封展旗, 杨同 卫,编著,经济管理出版社,p169-171,2000。

疫苗的主要作用在于预防疾病。现在也有应用疫苗来治疗疾病。所以,针对正常的人体和动物体而言,疫苗的作用在于增强机体的防病和抗病能力,起到预防疾病的目的;对于患有某种疾病或者病患的人体和动物体而言,疫苗的作用在于诱导机体产生针对特定致病因系的反应,达到消除病灶,治疗疾病或者病患的

目的。可以认为疫苗就是通过影响机体的免疫系统达到预防,或者治疗,或者预防和治疗疾病或者病患的物质的总称。

动物体内细胞分裂调节失控而无限增殖的细胞称为肿瘤细胞(tumor cell)。具有转移能力的肿瘤称为恶性肿瘤(malignancy),上皮组织的恶性肿瘤称为癌。参见,细胞生物学,翟中和等,主编,高等教育出版社,p423-427,2000(2001 重印)。对于肿瘤的起源和宿主对于肿瘤的应答已经进行了大量的基础研究和临床应用研究。关于起源,已经显示大量的环境因素对于动物具有致癌性和致突变性。有些肿瘤事实上与暴露于某种特定的物质有关(例如石棉与造船厂工人的间皮瘤)。已知病毒也可以诱发动物肿瘤,例如乙型肝炎病毒与肝癌有关。宿主免疫应答在许多情况下产生以抵抗肿瘤,而在某些情况下可能是保护性的。

肿瘤细胞和正常细胞可以根据其抗原的质和量的差异区分开来。肿瘤细胞的抗原,特别是肿瘤特异抗原为肿瘤细胞特有,成为肿瘤预防和治疗中的研究重点。

用于肿瘤治疗的肿瘤疫苗(cancer vaccine)尤其具有重要的意义。这种治疗性疫苗不同于传统的预防性疫苗,主要用于治疗已罹患癌症的病人,其目的以激发患者机体对肿瘤的特异性免疫应答,最终达到有效的排斥。研制和开发新型肿瘤疫苗已成为近年国际上肿瘤免疫治疗的热点,主要包括肿瘤细胞疫苗、基因修饰疫苗、多肽瘤苗和基因/DNA 疫苗等。

肿瘤细胞疫苗是将病人或者患病动物的肿瘤细胞通过物理或者化学方法处理后的具有治疗或者辅助治疗作用的完整的死细胞。处理方法可以是 X 光照射,有机溶剂处理等。这种疫苗被通过注射等方法施用于机体后,可以诱导或者增强病人对其肿瘤的免疫力。基因修饰疫苗、多肽瘤苗和基因/DNA 疫苗也均是通过利用肿瘤抗原及其片段,或者编码肿瘤抗原及其片段的多核苷酸,以及含有这些多核苷酸的载体或者细胞对于肿瘤进行治疗或者辅助治疗的疫苗。

如上所述,疫苗在疾病预防和/或治疗中具有重要作用。肿瘤作为一种严重的动物体细胞异常生长现象,随着环境污染的加剧,发生频率有所增强。因此开发新的疫苗,特别是肿瘤疫苗是必须的。本发明提供了一种制备疫苗的新方法,提供了一种新型肿瘤疫苗—EGFR 分子疫苗(EGFR molecular vaccine),满足了上述需要。

发明概述

第一个方面,本发明提供了一种制备疫苗的方法。该方法包括:

- 1) 分析特定病原体的特异抗原;
- 2) 获得编码特异抗原的多核苷酸序列;
- 3)获得与该多核苷酸序列有足够差异的多核苷酸序列:
- 4)利用步骤3)中所得的多核苷酸序列制备疫苗。

第二个发面,本发明提供了一种制备肿瘤疫苗的方法。该方法包括:

- 1) 分析动物体内的肿瘤细胞的特异抗原;
- 2) 获得编码特异抗原的多核苷酸序列;
- 3) 获得与该多核苷酸序列有足够差异的多核苷酸序列;
- 4) 利用步骤 3) 中所得的多核苷酸序列制备疫苗。 第三个方面,本发明提供了一种制备 EGFR 疫苗的方法。该方法包括:
- 1) 分析动物体内的肿瘤细胞的 EGFR 抗原;
- 2) 获得编码特异抗原的多核苷酸序列;
- 3) 获得与该多核苷酸序列有足够差异的多核苷酸序列;
- 4) 利用步骤 3) 中所得的多核苷酸序列制备疫苗。

第四个方面,本发明提供了一种核酸疫苗,其中含有与编码一种病原体上的一种特定抗原的多核苷酸序列有一定的差异,而且其同源性需要达到一定数值的多核苷酸序列。疫苗中所含多核苷酸序列与病原体中该特定抗原的多核苷酸序列所编码的多肽的同源性为 30-95%。

第五个方面,本发明提供了一种核酸疫苗,其中含有与编码一种病原体上的两种或者两种以上特定抗原的多核苷酸序列有一定的差异,而且其同源性分别要达到一定数值的两种或者两种以上多核苷酸序列。疫苗中所含多核苷酸序列与病原体中相应的特定抗原的多核苷酸序列所编码的多肽的同源性为 30-95%。

第六个方面,本发明提供了一种蛋白质或者多肽疫苗,其中含有与一种病原体上的一种特定抗原的蛋白质部分中的氨基酸序列有一定的差异,而且其同源性为 30-95%的蛋白质或者多肽分子。

第七个方面,本发明提供了一种蛋白质或者多肽疫苗,其中含有与一种病原体上的两种或者两种以上特定抗原的蛋白质部分中的氨基酸序列有一定的差异,而且其同源性为 30-95%的两种或者两种以上蛋白质或者多肽分子。

第八个方面,本发明提供了一种表皮生长因子受体(EGFR)核酸疫苗,其中含有与编码一种生物体内的 EGFR 分子的基因中的核苷酸序列有一定的差异,而且其同源性需要达到一定数值的一种多核苷酸序列。疫苗中所含多核苷酸序列编码的表皮生长因子受体与该生物体内的表皮生长因子受体中的氨基酸序列的同源性为 30-95%。

第九个方面,本发明提供了一种表皮生长因子受体(EGFR)核酸疫苗,其中含有与编码一种生物体内的 EGFR 分子的基因中的核苷酸序列有一定的差异,而且其同源性需要达到一定数值的两种或者两种以上的多核苷酸序列。疫苗中所含多核苷酸序列编码的表皮生长因子受体与该生物体内的表皮生长因子受体中的氨基酸序列的同源性为 30-95%。疫苗中所含的两种或者两种以上的多核苷酸序列可以具有不同的来源,其与该生物体内的 EGFR 分子的基因中的核苷酸序列的同源性可以不同。

第十个方面,本发明提供了一种表皮生长因子受体(EGFR)蛋白质或者多肽

疫苗,其中含有与一种生物体内的 EGFR 分子的变体。疫苗中所含表皮生长因子 受体的氨基酸序列与该生物体内的表皮生长因子受体中的氨基酸序列的同源性为 30-95%。

第十个方面,本发明提供了一种表皮生长因子受体(EGFR)蛋白质或者多肽疫苗,其中含有与两种或者两种以上的与生物体内 EGFR 分子不同的变体。疫苗中所含表皮生长因子受体的氨基酸序列与该生物体内的表皮生长因子受体中的氨基酸序列的同源性为 30-95%。

第十一个方面,本发明提供了的表皮生长因子受体核酸疫苗和表皮生长因子 受体蛋白质或者多肽疫苗用于肿瘤预防和治疗中的用途。

发明祥述

在此所用,"病原体"是指侵入生物体的外来生物体,包括细菌、病毒、真菌、原虫、蠕虫等,它们可以侵入另外的生物体中,导致一定的疾病或者疾病状态。

在此所用,"肿瘤细胞"是指生物体内发生了生长和分裂失控,脱离了衰老和死亡的正常途径的细胞。"肿瘤细胞"群构成肿瘤组织。在本发明中,术语"肿瘤"是以其广义而言,包括任何类型的细胞不正常的增殖,也指肿瘤组织或者肿瘤细胞,需要对这种增殖予以遏制或者将这些细胞以及组织予以抑制或者消除。

在此所用,"生物体"是指动物体,尤其指"人体"。对于存在有免疫系统的动物,本发明的方法和疫苗均可以适用。

可以利用本技术领域的基础研究结果或者已知的方法确定病原体或者肿瘤细胞中的特定抗原作为制备本发明所述疫苗的靶标。由于特定病原体或者体内的异常细胞具有特定的标记分子,比如它们的特定抗原,所以,以这些分子作为靶标,可以设计疫苗来激发生物体针对这些有害物质、生物或者细胞的免疫反应,达到防病和治病的目的。

从 GenBank 等公开的数据库,或者众多的网站上可以找到相应的有用抗原的基因序列,或者其氨基酸序列。根据中心法则,利用氨基酸序列或者基因序列进行推导所得的序列也可以应用于本发明中。有用的网站包括,但不限于,http://www.sanger.ac.uk/Projects/Microbes.

http://bioweb.pasteur.fr/Genoist/TubercuList.

在本发明中,使用的针对靶抗原的核酸序列或者多肽的氨基酸序列不同于编码该靶抗原的蛋白质或者多肽部分的核酸序列或者其氨基酸序列。其同源性以编码产物而言为 30-95%。

同源性通常用序列分析软件来测定。在上下文中对于两种多肽序列,术语"同源性"指相同或具有特定百分比的氨基酸残基的两个或多个序列,当在同一比较窗口或指定区域内尽可能一致地比较或对齐时,所述氨基酸残基是相同的,这正如用大量序列比较算法或通过人工对齐和视觉观察所测定那样。

对于序列比较,通常一个序列作为参考序列,测试序列与其进行比较。当使 用序列比较算法时,将测试序列和参考序列输入计算机,指定序列算法程序参数。 然后用序列比较算法基于程序参数计算测试序列相对于参考序列的序列同源性百 分比。在本发明中,某种选定的抗原中的氨基酸序列作为参考序列,疫苗中核苷 酸序列的编码序列或者疫苗中的蛋白质或者多肽中的氨基酸序列就是测试序列, 该测试序列与参考序列的同源性是本发明的一个方面。

如果将靶抗原的核酸序列或者多肽的氨基酸序列看作"母序列或者参考序列",那么本发明所用的核酸序列或者多肽的氨基酸序列可以看作"子序列或者衍生序列"。

"母序列或者参考序列"的来源可以是现有技术,比如上面提到的数据库,网站,公开的发明和论文等。它们也可以来源于对特定抗原,不论微生物抗原还是体内肿瘤的抗原的研究。可以是对基因组,染色体,蛋白质或者多肽,mRNA,DNA分子进行研究和分析获得母序列或者参考序列或者其序列信息。

"子序列或者衍生序列"是由"母序列或者参考序列"演变而来的序列,它 们与"母序列或者参考序列"有一定的差异,但是又不是完全不同。"子序列或者 衍生序列"可以是自然界已经存在的与"母序列或者参考序列"具有结构上关联 的序列,也可以是通过人工方法得到的序列。前者是指不同生物或者生物个体内 存在的编码同源蛋白的序列。比如,人表皮生长因子受体的编码序列和小鼠表皮 生长因子受体的编码序列,可以将它们看作本发明所称"母序列"与"子序列" 的关系,或者"参考序列"与"衍生序列"的关系。这种称呼可以相互交换。比 如将小鼠表皮生长因子受体的编码序列看作"母序列",则人表皮生长因子受体的 编码序列被看作"子序列";或者人表皮生长因子受体的编码序列被看作"母序列", 小鼠表皮生长因子受体的编码序列看作"子序列"。也可以以"母序列或者参考序 列"为起始物质,经过突变或者修饰得到"子序列或者衍生序列"。还可以通过人 工合成方法得到与"母序列或者参考序列"有点差异的"子序列或者衍生序列"。 通过检索有关数据库,在计算机上运行有关程序得到"母序列或者参考序列"的 "子序列或者衍生序列",然后通过人工合成得到具有"子序列或者衍生序列"的 核酸、蛋白质以及其片段的物质。通过筛选有关分子文库,也可以得到本发明所 称"子序列或者衍生序列"。具有"子序列或者衍生序列"的核酸,核酸片段,寡 核苷酸,多核苷酸以及具有"子序列或者衍生序列"的蛋白质,多肽,及其片段 可以用于本发明的实施中。在本发明实施中,对这些具有"子序列或者衍生序列" 的核酸,核酸片段,寡核苷酸,多核苷酸以及具有"子序列或者衍生序列"的蛋 白质,多肽,及其片段可以进行各种修饰。也可以将它们包括在适当的载体或者 细胞或者病毒中,达到本发明的发明目的。上述用于本发明实施中的各种物质可 以是分离的,可以是纯化的,它们之间也可以是混合的,还可以混合进一定物质, 比如佐剂。

此处所用,短语"核酸"或"核酸序列"指寡核苷酸、核苷酸、多核苷酸,或指寡核苷酸、核苷酸、多核苷酸中任一种的片段,指基因组或合成来源的 DNA或 RNA,指肽核酸(PNA),或者指任何天然或合成来源的类似 DNA 或类似 RNA的物质。

特定多肽或蛋白质的"编码序列"或"编码特定多肽或蛋白质的核苷酸序列" 是当在置于适当调节序列控制下时被转录或翻译到多肽或蛋白质中的核酸序列。

术语"基因"指涉及产生多肽链的 DNA 片段;包括编码区之前和之后的区(前导区和非转录尾区),以及适当的时候,包括个体编码片段(外显子)之间的间插序列(内含子)。

正如此处所用,"氨基酸"或"氨基酸序列"指寡肽、肽、多肽或蛋白序列,或指寡肽、肽、多肽或蛋白序列中任意一种的片段、部分或亚单位,指天然分子或合成分子。

正如此处所用,术语"多肽"指通过肽键或修饰的肽键即肽等排物(peptide isostere) 彼此连接在一起的氨基酸,并且可以含有除了20个由基因编码的氨基酸 以外的修饰氨基酸。多肽可以通过任一种天然方法修饰,如翻译后加工,或通过 本技术领域已知的化学修饰技术修饰。修饰可以发生在多肽中的任何地方,包括 肽骨架、氨基酸侧链和氨基末端或羧基末端。应该意识到, 在给定的多肽中, 相 同类型的修饰可以在数个位点以相同或变化的程度存在。而且,给定的多肽可以 具有许多类型的修饰。这些修饰包括乙酰化作用、酰化作用、ADP-核糖基化作用、 酰胺化作用、黄素的共价附着、亚铁血红素部分的共价附着、核苷酸或核苷酸衍 生物的共价附着、脂质或脂质衍生物的共价附着、交联环化、二硫键形成、脱甲 基作用、共价交联的形成、半胱氨酸的形成、焦谷氨酸的形成、甲酰化作用、γ-羧化作用、糖基化作用、GPI 锚形成、羟基化作用、碘化作用、甲基化作用、豆蔻 酰化、氧化、蛋白水解加工、磷酸化作用、异戊二烯化、外消旋作用、硒化作用、 硫酸化作用、t-RNA 介导的将氨基酸加入到蛋白质的过程如精氨酰化。(参考 Creighton, T.E., Proteins-structure and Molecular Properties 第二版, W.H. Freeman and Company, New York (1993); Posttranslational Covalent Modification of Proteins, B.C. Johnson 编著, Academic Press, New York, 1-12 页(1983))。

正如此处所用,术语"分离的"指物质离开其原始环境(例如,如果是天然存在的,则离开其天然环境)。例如,存在于活的动物体内的天然存在的多核苷酸或多肽不是分离的,但从天然系统中的一些或全部共存物质中分离出的相同多核苷酸或多肽是分离的。这样的多核苷酸可以是载体的一部分,和/或这样的多核苷酸或多肽可以是组合物的一部分,它们仍然是分离的,原因在于这些载体或组合物不是天然环境的一部分。

正如此处所用,术语"纯化的"不要求完全纯化;更确切地说,该术语是一个相对定义。

正如此处所用,术语"重组的"指核酸与"骨架"核酸相邻,而在其天然环境中与"骨架"核酸不是相邻的。根据本发明的骨架分子包括核酸,如表达载体、自我复制核酸、病毒、整合核酸和其它用于维持或操纵相关核酸插入物的载体或核酸。

"重组的"多肽或蛋白质指通过重组 DNA 技术产生的多肽或蛋白质;即,通 过编码期望多肽或蛋白质的外源 DNA 构建物转化的细胞产生的多肽或蛋白质。"合 成的"多肽或蛋白质是那些通过化学合成制备的多肽或蛋白质。也可以固相化学 肽合成方法来合成本发明的多肽或片段。这些方法自从 20 世纪 60 年代早期在本 技术领域就已经已知 (Merrifield, R. B., J. Am. Chem. Soc., 85:2149-2154, 1963) (也 可以参见 Stewart, J. M.和 Young, J. D., Solid Phase Peptide Synthesis, 第二版, Pierce Chemical Co., Rockford, I11, 11-12页)),并且最近已经被用于可通过商业途径获得 的实验室肽设计和合成试剂盒(Cambridge Research Biochemicals)。这些可通过商 业途径获得的实验室试剂盒通常使用了 H. M. Geysen 等人, Proc. Natl. Acad. Sci., USA, 81:3998(1984)的教导, 在大量"棒"或"针"的尖端上提供合成肽, 所有棒 或针与单个板子相连接。当使用这样的系统时,一个具有棒或针的板子被翻转, 且插入到具有相应孔或容器的第二个板子中,所述孔或容器含有将适当氨基酸附 着或固定到针或棒尖端的溶液。通过重复这一处理步骤,即将棒或针的尖端翻转 且插入到适当溶液中,氨基酸就被构建到期望肽中。此外,可以得到数量众多的 FMOC 肽合成系统。例如,多肽或片段的装配可以使用 Applied Biosystems, Inc. 提 供的 Model 431A 自动肽合成仪在固体载体上进行。该肽合成仪提供了现成获取本 发明所述肽,或者通过直接合成或者通过合成一系列片段,这些片段可以使用其 它已知技术偶联。

当起动启动子转录的 RNA 聚合酶将编码序列转录到 mRNA 中时,启动子序列与编码序列是"可操纵连接"的。

"质粒"由前面的小写字母"p",和/或其后的大写字母和/或数字标明。此处的起始质粒或者通过商业途径获得,是不受限制地公开获得,或者可以用与公开方法一致的方法从可获得的质粒构建而来。另外,与此处所描述的质粒等效的质粒在本领域是已知的,对于普通技术人员而言是明了的。在本发明中,构建了一些典型的质粒,参考附图 2。这些质粒是实施本发明的一种形式。

"寡核苷酸"或者指一个单链多脱氧核苷酸,或者指两个互补的多脱氧核苷酸链,它们可以化学合成。这样化学合成的寡核苷酸没有 5'磷酸,因此在没有用 ATP 添加磷酸和存在激酶的情况下,不会与另一个寡核苷酸连接。合成寡核苷酸将与没有发生去磷酸化作用的片段连接。

正如此处所用,"片段"是天然存在的蛋白质的一部分,其可以以至少两种不同的构象存在。片段可以具有与天然蛋白质相同或基本上相同的氨基酸序列。"基本上相同"指氨基酸序列大部分是相同的,但不是完全相同。

术语"变体"指在一个或多个碱基对、密码子、内含子、外显子或氨基酸残基(分别地)被修饰而不同于本发明的"母序列或者参考序列"。变体可以通过很多方法产生,这些方法包括,例如易错 PCR、改组、寡核苷酸介导的诱变、装配 PCR、有性 PCR 诱变、体内诱变、盒式诱变、递归集团诱变(recursive ensemble mutagenesis)、指数集团诱变(exponential ensemble mutagenesis)、位点特异性诱变、基因再装配、GSSM 及其任意组合。

从又一个方面来说,本发明所述的"子序列或者衍生序列"可以看作"母序列或者参考序列"的"变体"。因为它们在结构上与抗原中的"母序列或者参考序列"具有一定的相似性,而且又具有一定的差异。所以含有"子序列或者衍生序列"的物质可以诱发机体针对该抗原的免疫反应,尤其可以打破机体对该抗原业已产生的耐受性。其实,真是由于本发明所述疫苗中所用物质与抗原结构有一定相同性,机体发动的针对本发明疫苗中的物质的免疫反应就会交叉作用于该抗原。同时因为本发明所述疫苗中所用物质与抗原结构有一定差异,所以本发明的疫苗特别适用于诱发机体针对产生耐受性的抗原进行反应。

以下以 EGFR 分子疫苗为例来说明本发明的方法。事实上,本发明并不限于 EGFR 分子疫苗,本技术领域的技术人员根据本发明的教导可以方便地将本发明的 方法用于其他疫苗的制备中。本发明更重要的价值在于提出了一种制备疫苗,特别是打破机体耐受性的方法;尤其在于肿瘤治疗上有重要价值。

EGFR 分子疫苗就是利用经现代生物工程技术改造进化的或在生物进化过程中形成的来源于人或其他异种生物的同源 EGFR 分子作为抗原而构建的各种新生物技术疫苗,包括重组蛋白疫苗、重组基因疫苗、重组病毒疫苗、基因修饰疫苗和稳定转化共生菌。这是一种新型概念的肿瘤疫苗。

同源 EGFR 分子就是指与人 EGFR 分子在氨基酸或核苷酸水平上存在不同程度的相同程度的其它物种来源(如小鼠、大鼠、鸡、鲐、果蝇等,见 SEQ ID NO 1-14)或人工进化的 EGFR 分子的总称。

人表皮生长因子受体(epidermal growth factor receptor,EGFR)是一种分子量为 170KD 的跨膜单链糖蛋白,由 1186 个氨基酸组成,具有酪氨酸蛋白激酶(PTK)活性。EGFR 由胞外配体结合区、跨膜疏水区、近膜区、PTK 活性区和受体 C 端尾肽五个部分组成。EGFR 有多种不同形式的转录方式(见 SEQ ID NO 1-5),还有多种三种突变体,其中主要的突变体是第 III 类 EGFRvIII(又称 ΔEGFR 或de2-7EGFR,见 SEQ ID NO 6),是恶性肿瘤细胞膜上所特有的,具有高度的肿瘤特异性。人 EGFR 分子是一类基因家族,除 EGFR (亦称 c-erbB1/HER1)外其它家族成员还包括 c-erbB2/HER2、c-erbB3/HER3 和 c-erbB4/HER4,它们分别编码膜蛋白 p185erbB2、p160 erbB3、p180 erbB4。虽然这些成员具有高度同源的氨基酸序列和相似的结构特征,但配体各不相同。本发明所涉及的肿瘤疫苗靶分子是EGFR(c-erbB1/HER1),不包括 c-erbB2/HER2、c-erbB3/HER3 和 c-erbB4/HER4。

EGFR 分子的配体 EGF 或 TGF-可通过自分泌(autocrine)或旁分泌(paracrine)等途径作用于 EGFR,使其 PTK 活化,经过一系列的信号传递,继而导致细胞的分裂增殖。

EGFR 广泛分布于正常的哺乳动物上皮细胞表面,平均每个细胞的受体数为5-10 万。多种肿瘤细胞如肺癌、乳腺癌、卵巢癌、结肠癌、前列腺癌、胃癌、膀胱癌、头颈部鳞癌和胶质瘤等,都过量表达 EGFR,数量可达1-3×10⁶/细胞。以肺癌为例,EGFR 的过量表达还与肺癌的浸润、转移和预后密切相关。因此,EGFR 被认为是肿瘤治疗的一个理想的靶分子。目前,以 EGFR 为靶分子的治疗,主要表现为 EGFR 单克隆抗体和小分子化合物。此外,以 EGFRvIII 为靶分子的肿瘤肽疫苗、以及利用反义核酸技术进行基因治疗等取得了一些有益的进展。以 EGFR 分子为靶分子设计抗肿瘤分子疫苗,目前尚无任何文献报道和专利报告。

肿瘤细胞在其恶性转化、增殖的过程中要产生一种或多种肿瘤抗原。然而,在大多数情况下,肿瘤抗原的免疫原性较弱,并不足以引起机体的主动免疫反应。从免疫学的角度看,肿瘤细胞就是一种能不断表达"正常"抗原(基因过度表达)和/或"异常"抗原(基因修饰、突变或缺失等)的宿主体内"自身"组织细胞,因此肿瘤抗原可看作是自体抗原。在正常情况下,机体对自体抗原不产生免疫应答,即呈现出免疫耐受。事实上,自体抗原是宿主免疫系统必须耐受的最相关和最丰富的抗原。自体抗原耐受的诱导和维持是由多种机制介导的,它们能防止对正常组织的不适当破坏。然而,当机体隐蔽的抗原释放、或生物、物理与化学等因素使自体抗原发生改变等情况下,却可导致自体免疫反应,造成靶抗原所在的细胞、组织或器官的病理性损伤和功能障碍。这样,如果能使肿瘤细胞隐蔽的自体抗原释放或者使其发生某种改变的话,则可能因此诱导出机体对自身肿瘤细胞的特异性自体免疫反应,从而使肿瘤缩小乃至消退。如前文所述,EGFR 分子在多种肿瘤中过量表达,因此,EGFR 分子可看作是一种肿瘤抗原,也是一种自体抗原。因此,可对人 EGFR 抗原分子(见 SEQ ID NO 1-6)进行各种生物技术改造,制备成分子疫苗,从而诱导抗肿瘤免疫反应,达到抗肿瘤作用的目的。

生物在进化过程中,不同物种之间存在着具有不同程度同源性的大量分子,如 EGFR 分子就广泛存在于人、鼠、果蝇、鱼类、鸟类等生物,它们相互之间存在不同的同源性程度,人的 EGFR 分子在氨基酸水平上与鼠、鸡和果蝇的 EGFR 分子的同源性分别为 90%、64%和 32%。我们可以利用异种同源分子在进化过程中所形成的细微差别来打破免疫耐受、增强免疫原性、诱导肿瘤细胞的自体免疫反应进而达到抗肿瘤的目的。这其中的机制很可能是:由于在进化过程中同源基因的中性突变虽不导致其功能的丧失或改变,但却可能影响到或改变了其免疫应答的方式。当异种同源基因导入到受试对象体内表达出相应的异种同源蛋白,受试者将之识别为外来抗原,一方面产生相应的抗体或细胞毒性淋巴细胞来清除它,另一方面由于这些表达出的异种同源蛋白与受试者体内相应的蛋白分子存在某种

程度上的差异而与之产生非特异性的交叉反应,从而诱导自身免疫反应,打破机体对自身的这种蛋白的免疫耐受(见说明书附图1)。

如前文所述,人的 EGFR 与其他物种(如鼠、鸡、果蝇等)的 EGFR 在氨基酸水平上表现出不同程度的同源性,通过 BLAST 分析发现人的 EGFR 在氨基酸水平上与鼠、鸡和果蝇的同源性分别 90%、64%和 32%。我们利用异种同源 EGFR 分子产生的种与种之间的免疫交叉反应来诱导机体的主动免疫反应,克服自身肿瘤细胞的免疫耐受,通过其产生的抗体竞争性抑制 EGFR 与配体 EGF 或 TGF- 的结合,从而抑制肿瘤细胞的生长,此外还通过其所诱导的 CTL 来抑制肿瘤细胞的生长。

此外,现代生物工程技术的发展,如易错 PCR 技术、DNA"洗牌术"(DNA shuffling)、噬菌体展示技术等,为 EGFR 分子的人工基因直接进化 (gene directed evolution) 提供了技术支持。

本发明涉及抗肿瘤 EGFR 分子疫苗,包括自体 EGFR 分子疫苗 (autologous EGFR vaccine)、异种 EGFR 分子疫苗 (xenogeneic EGFR vaccine) 和基因直接进化 EGFR 分子疫苗 (directed evolution EGFR Vaccine)。

本发明主要涉及一种新型的肿瘤疫苗——EGFR 分子疫苗,它是利用经现代生物工程技术改造的或在生物进化过程中形成的来源于人或其他异种生物的同源EGFR 分子作为抗原而构建的各种新生物技术疫苗,包括蛋白疫苗、基因疫苗、病毒疫苗、基因修饰疫苗和稳定转化共生菌。EGFR 分子疫苗对各种过表达 EGFR 分子的实体瘤都具有抗肿瘤作用效应,其抗肿瘤作用机制在于: EGFR 分子作为免疫交叉抗原,能打破机体对自身 EGFR 分子的免疫耐受,诱导机体产生针对 EGFR 分子自身免疫样交叉反应。抗肿瘤 EGFR 分子疫苗也可以是利用纳米生物技术制备而成的纳米粒制剂。

一、 EGFR 分子的来源、选择与改造

本发明涉及的 EGFR 分子包括自体 EGFR 分子 (autologous EGFR molecule)和异种 EGFR 分子(xenogeneic EGFR molecule)和基因直接进化 EGFR 分子(directed evolution EGFR molecule)。

利用在生物进化过程中形成的或经现代生物工程技术改造的同源 EGFR 分子的差异性进行抗肿瘤免疫治疗是本发明的最大特征。

EGFR 在自然界中分布广泛,从哺乳动物(如人、鼠等)、鸟类(如鸡等)、鱼类(如鲐等)到低等生物(如线虫、果蝇等)都有 EGFR 的表达,这些生物来源的 EGFR 分子之间都存在一定的差异,其同源性程度在 30-100%之间。本发明所涉及的异种 EGFR 分子的同源性在 30-95%之间,主要涉及的生物物种是人、鼠、鸡和果蝇,在氨基酸水平上,人的 EGFR 分子与鼠、鸡和果蝇的 EGFR 分子的同源性分别为 90%、64%和 32%,人、鼠、鸡和果蝇的 EGFR 分子分别代表了本发明中所涉及的同源性程度的监测点。

自体 EGFR 分子由于存在免疫耐受,故免疫原性较弱。利用现代生物工程技术可对自体 EGFR 分子进行人工直接进化(gene directed evolution)和改造,增强 EGFR 分子作为抗原的免疫原性。通常是利用易错 PCR、随机引物延伸技术和 DNA"洗牌"术(DNA shuffling)等对自体 EGFR 分子进行人工突变,建立基因突变库,然后使用噬菌体展示技术(phage display)、核糖体展示技术(ribosome display)等进行筛选,从而得到免疫原性强的 EGFR 分子。

此外,利用细菌、病毒等不同生物的表达修饰系统的差异,也可以在蛋白质水平上对自体 EGFR 分子进行人工改造,增强自体 EGFR 的免疫原性。

二、 EGFR 重组基因疫苗

基因疫苗是利用现代分子生物学技术构建制备的以核酸为基础的新型疫苗,主要是 DNA 疫苗。

本发明的 EGFR 分子疫苗之一就是 DNA 疫苗。依据 GenBank 等各公开的数据库所收藏的各种生物来源(如人、小鼠、大鼠、鸡、鲐、果蝇等,见 SEQ ID NO 1-14)的 EGFR 分子的序列(包括基因、cDNA、mRNA 和氨基酸序列)按常规方法设计引物或探针,用 PCR、RT-PCR、杂交等技术,从各种商品化的基因文库、cDNA 文库或各种细胞系、组织等中克隆分离出不同种属生物来源的 EGFR 分子的胞外区段的 cDNA(发明人发现 EGFR 胞外区段才是引起免疫反应的反应域),或进一步用基因直接进化技术筛选到的具有强免疫原性的 EGFR 分子。各种来源的 EGFR cDNA 的胞外段序列经测序鉴定后,用分子克隆技术构建其真核生物表达质粒系统,然后转染入 CHO 细胞系,观察并检测其表达 EGFR 的情况和水平。各重组 EGFR 分子的真核生物表达质粒可经过限制性酶切分析、SDS-PAGE 和Western Blot 等进行鉴定、确证。用碱法抽提经过鉴定的重组 EGFR 分子表达质粒,用超离心、超滤等方法去除大肠杆菌内毒素,即得到纯净的质重组粒 DNA,这些质粒 DNA 即可作为 DNA 疫苗进行免疫。

DNA 分子疫苗的代表性质粒图谱如图 2A 所示,其具体构建过程简要叙述如下: 依据 GenBank 等各公开的数据库所收藏的人、小鼠、鸡、的 EGFR 分子的 cDNA 序列(分别对应于 SEQ ID NO 1-5,7-9,19)设计 PCR 引物(人的引物为: 5'GACCATG GAGGAAAAGAAAGTTTGC 3', 5'ACGAATTCTTAGGACGGGATCTTAGGCCCA 3'; 小鼠的引物为: 5'GACCATGGAGGAAAAGAAAGTCTGC 3', 5'ACGAATTC TTAATAGATGGTATCTTTGGC 3'; 鸡的引物为: 5'GACCATGGAGGAGAAAGAA AGTTTGTC 3', 5'ACGAATTCTTAAGATGGAGTTTTGGAGCC 3'), 分别以人肺癌细胞株 A431、小鼠肺癌细胞株 LL2 和鸡胚的总 RNA 为模板进行 RT-PCR 扩增,电泳收集纯化扩增的 EGFR 片段(均为 1.9kb),然后进行 PCR 产物亚克隆。把 PCR 亚克隆测序确证后,用 Ncol 和 EcoRl 酶切,收集 1.9kb 片段并纯化,插入到用 Ncol 和 EcoRl 双酶切的 pORF-MCS (InvivoGen 公司) 载体,筛选重组质粒。候选的重组质粒经限制性酶切分析及 PCR 扩增等双重鉴定,命名为 pORF-hEGFR、

pORF-mEGFR 和 pORF-chEGFR。至于 pcDNA-hEGFR、pcDNA-mEGFR 和 pcDNA-chEGFR 的构建过程与上述方法一致 (pcDNA3.1(+)载体来源于 Invitrogen 公司),只是 PCR 引物略有改动,分别是:人的引物 5'GAGCTAGCATGGAGGAAA AGAAAGTTTGC 3', 5'CACTCGAGTTAGGACGGATCTTAGGCCCA 3'; 小鼠的引物为: 5'GAGCTAGCATGGAGGAAAAGAAAGTCTGC 3', 5'CACTCGAGTTAATAGATGGTATCTTTGGC 3'; 鸡的引物为: 5'GAGCTAGCATGGAGGAGAA GAAAGTTTGTC 3', 5'CACTCG AGTTAAGATGGAGTTTTGGAGCC 3'。

用人的EGFR基因胞外区段构建和制备DNA疫苗免疫Lewis 肺癌的模型小鼠,发现免疫后的第 8 周,注射人 EGFR 疫苗的小鼠生存率为 78%,明显高于注射过小鼠 EGFR 疫苗(25%)和对照实验动物(10%~15%),同时并未发现模型小鼠的肺、肝、心、肾等有病理性改变,进一步的研究表明诱导的自体免疫反应主要依赖于CD4⁺T 淋巴细胞,CTL 活性检测没有发现靶细胞特异性的杀伤效应。免疫组化结果显示肿瘤组织有自身抗体沉着,而肺、肝等组织则没有,自身反应抗体主要为IgG。

以 EGFR 分子为模板设计的各种反义 RNA 和 RNAi 可看作是 EGFR 重组基因 疫苗的特例,它主要不是通过提高 EGFR 分子的免疫原性,诱导产生抗 EGFR 抗体和特异性的 CTL,进而阻断 EGFR 信号通路,诱导肿瘤细胞凋亡,抑制肿瘤细胞的生长和扩散,而是在 DNA 和 RNA 水平上直接抑制和阻断 EGFR 分子的表达。

三、 EGFR 重组蛋白疫苗

蛋白疫苗是比较传统形式的疫苗,但蛋白质有较好的免疫原性。本发明的 EGFR 分子疫苗之一就是重组蛋白疫苗,包括以各种大肠杆菌重组表达载体、酵母 重组表达载体、棒状病毒重组表达载体等表达系统表达出的重组蛋白所构建而成 的疫苗。

利用分子克隆技术(如 PCR、RT-PCR、杂交等技术)从各种商品化的基因文库、cDNA 文库或各种细胞系、组织等中克隆分离出不同种属生物来源的 EGFR 分子的胞外区段的 cDNA,或进一步用基因直接进化技术筛选到的具有强免疫原性的 EGFR 分子,然后用分子克隆技术构建其原核生物表达质粒系统,转化合适的大肠杆菌宿主,观察并检测其表达 EGFR 的情况和水平。各重组 EGFR 分子的表达质粒可经过限制性酶切分析、SDS-PAGE 和 Western Blot 等进行鉴定、确证。经确证的重组 EGFR 分子原核表达质粒转化 E.coli,大量培养重组菌落,低温离心收集菌体,菌体经 PBS 重悬后用超声法破碎细胞,用离子交换层析、亲和层析等方法分离纯化重组 EGFR 蛋白,此重组的 EGFR 蛋白质即可作为蛋白疫苗进行免疫。

E.coli 表达的重组 EGFR 蛋白疫苗的代表性质粒图谱如图 2B 所示,其具体构建过程简要叙述如下:依据 GenBank 等各公开的数据库所收藏的人、小鼠、鸡、的 EGFR 分子的 cDNA 序列(分别对应于 SEQ ID NO 1-5,7-9,19)设计 PCR 引物(人的引物为:5'GACCATGGAGGAAAAGAAAGTTTGC 3',5'ACAGATCTAGG

ACGGGATCTTAGGCCCA 3'; 小鼠的引物为: 5'GACCATGGAGGAAAAGAA AGTCTGC 3', 5'ACAGATCTATAGATGGTATCTTTGGC 3'; 鸡的引物为: 5'GACCATGGAGGAGAAGAAAGTTTGTC 3', 5'ACAGATCTAGATGGAGTTTT GGAGCC 3'), 分别以 pORF-hEGFR、pORF-mEGFR 和 pORF-chEGFR 等为模板进行 PCR 扩增,电泳收集纯化扩增的 EGFR 片段(均为 1.9kb),然后进行 PCR 产物亚克隆。把 PCR 亚克隆测序确证后,用 NcoI 和 BglII 酶切,收集 1.9kb 片段并纯化,插入到用 NcoI 和 BglII 双酶切的 pQE60(QIAGEN 公司)载体,筛选重组质粒。候选的重组质粒经限制性酶切分析及 PCR 扩增等双重鉴定,命名为pQE-hEGFR、pQE-mEGFR 和 pQE-chEGFR。

除上述利用大肠杆菌重组表达系统表达各种来源的 EGFR 分子外,还可以利用酵母重组表达系统、棒状病毒重组表达系统等表达 EGFR 重组蛋白,从而制备重组蛋白疫苗。EGFR 重组蛋白疫苗的构建流程图见图 3。

酵母表达的重组 EGFR 蛋白疫苗的代表性质粒图谱如图 2C 所示, 其具体构建 过程简要叙述如下: 依据 GenBank 等各公开的数据库所收藏的人、小鼠、鸡、的 EGFR 分子的 cDNA 序列 (分别对应于 SEQ ID NO 1-5,7-9,19) 设计 PCR 引物 (人 的引物为: 5'ATACTCGAGAAAAGAGAGGGAAAAGAAAG 3', 5' GCTCTAGAATGGCACAGGTGGCACA 3'; 小鼠的引物为: 5'ATGCTCGAGAAAA GAGAGTTGGAGGAAAAGAAGTC 3', 5'AAGCGGCCGCCATAGATGGTATCT TTG 3'; 鸡的引物为: 5'ATACTCGAGAAA AGAGAGGTGGAGGAGAAGAAG 3', 5'CGTCTAGAAGATGGAGTTTTGGAG 3'), 分别以 pORF-hEGFR、 pORF-mEGFR 和 pORF-chEGFR 等为模板进行 PCR 扩增,电泳收集纯化扩增的 EGFR 片段(均为 1.9kb), 然后进行 PCR 产物亚克隆。把 PCR 亚克隆测序确证后, 用 XhoI 和 XbaI(对小鼠的克隆为 XhoI 和 NotI)酶切, 收集 1.9kb 片段并纯化, 插入 到用 XhoI 和 XbaI 双酶切(对小鼠的克隆为 XhoI 和 NotI 双酶切)的 pPICZ A (Invitrogen 公司) 载体,转化 E.coli 筛选重组质粒。候选的重组质粒经限制性酶 切分析及 PCR 扩增等双重鉴定,命名为酵母表达质粒 pYE-hEGFR、pYE-mEGFR 和 pYE-chEGFR。这些酵母表达质粒经 PmeI 酶切线性化后,用电穿孔法转化酵母 菌株 X33 或 GS115, 用 Zeocin 抗性筛选稳定转化子, 在 MMH (Minimal Methanol with histidine, MMH) 和 MDH (Minimal Dextrose with histidine, MDH) 琼脂平板 上鉴定和挑选 Mut⁺转化子。挑选 6-10 个 Mut⁺转化子进行小规模表达,用 SDS-PAGE、Western Blot、ELISA 等方法对所表达的重组蛋白进行鉴定。选择其 中表达效率最高的 Mut[†]转化子做大规模表达,建立各级酵母表达种子库。重组菌 株大量摇瓶培养或发酵,低温离心收集菌体,菌体经 PBS 重悬后用超声法破碎细 胞,用离子交换层析、亲和层析等方法分离纯化重组 EGFR 蛋白,此重组的 EGFR 蛋白质即可作为蛋白疫苗进行免疫。

同样地,也可用其他的酵母表达系统构建类似的酵母重组表达质粒。

重组蛋白疫苗具有比 DNA 疫苗更强的诱导免疫交叉反应的作用,产生高滴度的抗 EGFR 抗体以及特异性的 CTL,从而抑制肿瘤细胞的生长和转移。

四、 EGFR 重组病毒疫苗

重组病毒也是一种良好的分子疫苗转运系统,这些重组病毒表达系统构建的分子疫苗包括重组腺病毒疫苗、Lentivirus 疫苗、腺病毒相关病毒疫苗、逆转录病毒疫苗、痘苗病毒疫苗和单纯疱疹病毒疫苗。

腺病毒载体是目前在肿瘤基因治疗中最有效的载体之一,它具有滴度高、安全性好、能感染分裂或不分裂细胞、不整合插入染色体等优点。此外,腺病毒还具有较强的免疫原性,这对于基因治疗也许是一个缺点,但在基因免疫治疗中却可能是一个很大的优点。重组腺病毒疫苗是 EGFR 重组病毒疫苗最重要的一种。同前文所述,首先克隆出各种同种、异种或基因直接进化的 EGFR cDNA,然后用分子生物学技术构建其重组腺病毒表达载体,转染 293 细胞,即得到重组的腺病毒。重组的腺病毒经 PCR、Western Blot 等加以确证。利用 293 细胞大量扩增经确证的 EGFR 重组腺病毒疫苗,用超离心、超滤等技术分离、纯化重组腺病毒,此经纯化的 EGFR 重组腺病毒即可作为疫苗进行免疫。EGFR 重组腺病毒疫苗的构建流程示意图见图 4。

EGFR 重组腺病毒是利用 AdEasy 系统构建而成的, 其具体构建过程简要叙述 如下: 依据 GenBank 等各公开的数据库所收藏的人、小鼠、鸡、的 EGFR 分子的 cDNA 序列(分别对应于 SEQ ID NO 1-5,7-9,19)设计 PCR 引物(人的引物为: 5'GAAGATCTATGGAGGAAAAGAAAGTTTGC 3', 5'ACGATATCTTAAGGACGG GATCTTAGGCCCA 3'; 小鼠的引物为: 5'GAAGATCTATGGAGGAAAAGAAAG TCTGC 3', 5'ACGATATCTTAATAGATGGTATCTTTGGC 3'; 鸡的引物为: 5'GAAGATCTATGGAGGAGAAGAAGTTTGTC 5'ACGATATCTTAAGATGGA GTTTTGGAGCC 3'), 分别以 pORF-hEGFR、 pORF-mEGFR 和 pORF-chEGFR 等为模板进行 PCR 扩增,电泳收集纯化扩增的 EGFR 片段(均为 1.9kb), 然后进行 PCR 产物亚克隆。把 PCR 亚克隆测序确证后, 用 Bg/II 和 EcoRV 酶切, 收集 1.9kb 片段并纯化, 插入到用 Bg/II 和 EcoRV 双酶切 的 pShuttle-CMV(Quantum Biotechnologies 公司)载体,筛选重组质粒。候选的重 组质粒经限制性酶切分析及 PCR 扩增等双重鉴定,命名为腺病毒穿梭表达质粒 pShuttle-hEGFR、pShuttle-mEGFR 和 pShuttle-chEGFR (图 2D)。分别将经 PmeI 酶切的腺病毒穿梭表达载体 pShuttle-EGFR 与包含腺病毒基因组的骨架载体 pAdEasy-1 或 pAdEasy-2 共转化 Ecoli BJ5183, 得到重组腺病毒载体质粒 pAd-hEGFR、pAd-mEGFR 和 pAd-chEGFR。这些重组腺病毒载体质粒经 PacI 酶 切后,用磷酸钙-DNA 共沉淀法转染入腺病毒包装细胞株 293 细胞中,得到相应的 重组腺病毒 Ad-hEGFR、Ad-mEGFR 和 Ad-chEGFR。用 PCR、Western blot 等确证 EGFR 基因构建入重组腺病毒载体中并能在真核细胞中得到正确有效的表达。

根据 EGFR 重组腺病毒疫苗中所包含腺病毒基因组的不同,又有 EGFR 重组 I 代腺病毒(腺病毒穿梭表达质粒 pShuttle-EGFR 与 pAdEasy-1 的重组体,命名为 Ad-hEGFR I、Ad-mEGFR I和 Ad-chEGFR I)疫苗和 EGFR 重组 II 代腺病毒(腺病毒穿梭表达载体 pShuttle-EGFR 与 pAdEasy-2 的重组体,命名为 Ad-hEGFR II、Ad-mEGFR II和 Ad-chEGFR II)疫苗之分。

此外,EGFR 重组腺病毒疫苗还可以用甘露聚糖 (Mannan)等进行靶向修饰。 EGFR 重组腺病毒疫苗由于腺病毒良好的基因导入性,能有效诱导机体产生抗 肿瘤免疫反应,抑制 EGFR 高表达肿瘤的生长。

Lentivirus 病毒载体是新一代的基因治疗载体,它来源于 HIV-1 的复制缺陷型 lentivirus,与传统的来源于的莫洛尼氏白血病毒(Moloney Leukemia Virus,MoMLV)的逆转录病毒载体不同,它对处于分裂和不分裂状态的哺乳动物细胞都能进行有效转染,且生物安全性更好。此外,与腺病毒载体不同的是,Lentivirus 病毒载体能整合到细胞染色体中,从而使导入的外源基因获得稳定、长久的表达。重组 Lentivirus 病毒疫苗也是 EGFR 重组病毒疫苗较重要的一种。同前文所述,首先克隆出各种同种、异种或基因直接进化的 EGFR cDNA,然后用分子生物学技术构建其重组 Lentivirus 表达载体,转染 293FT 细胞,即得到重组的 Lentivirus 病毒。重组的 Lentivirus 表达载体,转染 293FT 细胞,即得到重组的 Lentivirus 病毒。重组的 Lentivirus 病毒疫苗,用超离心、超滤等技术分离、纯化重组 Lentivirus 病毒,此经纯化的 EGFR 重组 Lentivirus 病毒即可作为疫苗进行免疫。EGFR 重组 Lentivirus 病毒疫苗的构建流程示意图见图 4B。

EGFR 重组 Lentivirus 病毒是利用 ViraPowerTM Lentiviral Gateway[®] Expression Kit (Invitrogen 公司)构建而成的,其具体构建过程简要叙述如下: 依据 GenBank 等各公开的数据库所收藏的人、小鼠、鸡、的 EGFR 分子的 cDNA 序列 (分别对 应于 SEQ ID NO 1-5,7-9,19) 设计 PCR 引物 (人的引物为: 5'GACCATGGAGGAA AAGAAAGTTTGC 3', 5'ACGATATCAGGACGGGATCTTAGGCCCA 3'; 小鼠的 引物为: 5'GACCATGGAGGAAAAGAAGTCTGC 3', 5'ACGATATCATAGATG GTATCTTTGGC 3'; 鸡的引物为: 5'GACCATGGAGGAGAAGAAGTTTGTC 3', 5'ACGATATCAGATGGAGTTTTGGAGCC 3'), 分别以 pORF-hEGFR、 pORF-mEGFR 和 pORF-chEGFR 等为模板进行 PCR 扩增, 电泳收集纯化扩增的 EGFR 片段(均为1.9kb), 然后进行 PCR 产物亚克隆。把 PCR 亚克隆测序确证后, 用 NcoI 和 EcoRV 酶切,收集 1.9kb 片段并纯化,插入到用 NcoI 和 EcoRV 双酶切 的 pENTR11 (Invitrogen 公司) 载体中,筛选重组质粒,命名为 pENTR-hEGFR、 pENTR-mEGFR 和 pENTR-chEGFR (图 2E)。分别将这些载体 pENTR-EGFR 与包 含 Lentivirus 病毒基因组的骨架载体 pLenti6/V5-DEST 共转化 E.coli DH5 , 得到 重组 Lentivirus 病毒载体质粒 pLenti-hEGFR、pLenti-mEGFR 和 pLenti-chEGFR(图 2E)。这些重组 Lentivirus 病毒载体质粒与包装混合物一起 (ViraPowerTM Packaging

Mix) 用磷酸钙-DNA 共沉淀法转染入 Lentivirus 病毒包装细胞株 293FT 细胞中,得到相应的重组 Lentivirus 病毒 Lenti-hEGFR、Lenti-mEGFR 和 Lenti-chEGFR。用 PCR、Western blot 等确证 EGFR 基因构建入重组 Lentivirus 病毒载体中并能在真核细胞中得到正确有效的表达。

五、 EGFR 分子疫苗的修饰和改进

前文的重组 EGFR 分子疫苗(包括重组 EGFR 基因疫苗、蛋白疫苗和病毒疫苗)只是基本形式,本发明还在高效性、特异性等方面有许多修饰和改进,这些改进包括:

1. EGFR 分子疫苗佐剂的选择

合适的佐剂是有效提高疫苗的免疫效应的良好载体,不同的疫苗形式有不同的佐剂。本发明 EGFR DNA 疫苗的佐剂主要是福氏佐剂和脂质体,EGFR 蛋白疫苗的佐剂主要是铝盐,EGFR 重组病毒疫苗通常不使用佐剂。

2. EGFR 基因修饰疫苗

基因修饰疫苗是指转染了人或其他异种生物的 EGFR 分子的细胞疫苗,包括使用稳定转化的各种肿瘤细胞株、血管内皮细胞和树突状细胞制备的细胞疫苗、用各种重组病毒感染的肿瘤细胞或肿瘤组织制备的疫苗。EGFR 基因修饰疫苗是EGFR 分子疫苗的一种特例形式。

前文已描述多种肿瘤包括肺癌、乳腺癌、卵巢癌、结肠癌、前列腺癌、胃癌、膀胱癌、头颈部鳞癌和胶质瘤等都高表达 EGFR,但这些肿瘤均对 EGFR 耐受,并不产生抗 EGFR 的免疫反应。但是,利用基因修饰疫苗可打破免疫耐受,产生抗 EGFR 免疫反应。用重组的各种来源的 EGFR 分子的真核表达质粒转染各种肿瘤细胞株(如肺癌细胞株 A431、乳腺癌细胞株 MCF7 等)、肿瘤血管内皮细胞等,筛选稳定表达 EGFR 分子的转化株,收获稳定转化株,用副甲醛固定法等制备细胞疫苗。此外,也可以用各种来源的 EGFR 构建的各种 EGFR 重组病毒疫苗感染各种肿瘤细胞株、肿瘤组织和肿瘤血管内皮细胞等,用副甲醛固定法等制备细胞疫苗。

3. EGFR 稳定转化共生菌

EGFR 分子疫苗还有活体疫苗形式,这就是 EGFR 稳定转化共生菌。

用各种来源的(包括自体的、异体的和基因直接进化的) EGFR 构建的原核生物重组表达质粒,转化肠道共生菌,如双岐杆菌等,筛选其稳定表达菌株。这些稳定表达的肠道共生菌菌株能持续分泌外源 EGFR 分子,诱导机体免疫反应,是一种活体形式的 EGFR 疫苗,它是 EGFR 分子疫苗的特例。

4. EGFR 分子疫苗的靶向纳米粒制剂

单纯的重组 EGFR 分子疫苗(包括重组 EGFR 基因疫苗、蛋白疫苗和病毒疫苗)依然有它的缺陷,其特异性不够,免疫原性仍有待提高。不发明利用纳米生物技术对 EGFR 分子疫苗进行靶向修饰,制备 EGFR 分子疫苗的靶向纳米粒制剂。

这些靶向纳米粒以EGFR 分子(包括 DNA 和蛋白质形式)为抗原靶分子,以生物性分子如脂质体、可降解性高分子生物材料如聚丙交酯-乙交酯 (poly DL-lactide-co-glycolide polymer, PLGA)等为纳米材料,以MIP-3 、MIP-3 等基因以及甘露聚糖 (Mannan)、Flt3-L 等为修饰基团,以树突状细胞为靶细胞构建而成。

本发明的 EGFR 分子疫苗靶向纳米粒制剂有多种形式,主要包括:①甘露糖化纳米粒(包括甘露糖化腺病毒重组 EGFR 疫苗、甘露糖化重组 EGFR 蛋白疫苗、甘露糖化脂质体重组 EGFR 基因疫苗、甘露糖化重组 EGFR 蛋白疫苗等),②基因靶向纳米粒(基因靶向纳米脂质体重组 EGFR 疫苗、基因靶向纳米 PLGA 重组 EGFR 疫苗、基因靶向重组 EGFR 腺病毒疫苗等),它们同时表达 EGFR 和 MIP-3 等基因,③肿瘤新生血管靶向腺病毒重组 EGFR 疫苗(RGD 修饰的腺病毒重组 EGFR 疫苗)。

本发明的纳米粒粒径通常在 500nm 以下,可分为三种规格 200-500nm、100-200nm 和 50-100nm,以 50-100nm 粒径的纳米粒的效果为最佳,纳米粒峰值在80nm 左右。

甘露糖化腺病毒重组 EGFR 疫苗的制备过程简要描述如下:用常规方法扩增 EGFR 重组腺病毒(I 代、II 代均可),层析或超离心法纯化重组腺病毒。将 70mg mannan(sigma)溶于 5ml 0.1M 的磷酸盐缓冲液(pH6.0)中,终浓度 14mg/ml,加 45ml 0.01M 高碘酸钠溶液,在 4℃下混合氧化 60 分钟,加入 10 1乙二醇,在 4℃下孵育 30 分钟,即得 Ox-M(Oxidative Mannan,Ox-M)混合物。将 Ox-M 混合物倒入用重碳酸盐缓冲液(pH6.0-9.0)平衡 Sephadex-G25 层析柱进行层析分离,OX-M 即被洗入 2ml 的空容器。将纯化的 Ox-M 与 1×10¹⁴ 腺病毒颗粒混合,室温过夜,即获得所需 Ox-M-腺病毒。在 Ox-M-腺病毒中加入 1mg/ml 硼氢化钠,室温放置 3 小时即得 Red-M-腺病毒(Reductive Mannan,Red-M)。Ox-M-腺病毒与Red-M-腺病毒经超滤脱盐、浓缩后,过滤细菌,小管分装,一80℃低温保存。此经纯化的甘露糖化重组 EGFR 腺病毒即可作为疫苗进行免疫。

本发明认为腺病毒颗粒的粒径在 80nm 左右,是天然的纳米粒。对腺病毒颗粒进行修饰,使腺病毒纤维蛋白(Adenoviral fiber protein)表达 RGD 三肽,该三肽对肿瘤血管内皮细胞有特异靶向性。用 RGD 修饰的腺病毒重组 EGFR 疫苗,可看作是天然的靶向纳米 EGFR 分子疫苗。

本发明利用 AdEasy 系统构建 RGD 修饰腺病毒的重组 EGFR 疫苗,图 5显示了 RGD 修饰的腺病毒重组 EGFR 疫苗的构建流程,其具体过程是: 腺病毒基因组骨架质粒 pAdEasy-1 和 pAdEasy-2 经限制性内切酶 SpeI(Sp)酶切后,用 T4 DNA聚合酶补平(filling, f) 末端,再用 PacI(P)酶切,分别电泳回收 6211bp 和 3579bp的片段,分别命名为 AdFiber I/Sp/f/P 和 AdFiber II/Sp/f/P,该片段包含了完整的腺病毒纤维蛋白基因。把 AdFiber I/Sp/f/P 和 AdFiber II/Sp/f/P 片段插入经 BamHI 酶

切一T4 DNA 聚合酶补平一PacI 酶切处理 (BamHI/filling /PacI-digested)的 pShuttle 载体,将所得的重组质粒分别命名为 pSh-AdFiber I 和 pSh-AdFiber II。 pSh-AdFiber I用 NheI 酶切一T4 DNA 聚合酶补平一KpnI 酶切处理 (NheI/filling/KpnI), 电泳回 收 2090 bp 的片段 AdFiber I/Nh/f/K,将该片段插入到经 SmaI/KpnI 双酶切的 pUC18 载体中,所得的重组质粒命名为 pUC-AdFiber I; 而 pSh-AdFiber II 用 AvrII 酶切一 T4 DNA 聚合酶补平-HindIII 酶切处理(AvrII/filling/HindIII),电泳回收 838 bp 的片段 AdFiber I/A/f/H,将该片段插入到经 Smal/HindIII 双酶切的 pUC18 载体中, 所得的重组质粒命名为 pUC-AdFiber II。设计一系列 PCR 引物以便以 pUC-AdFiber I 和 pUC-AdFiber II 为模板扩增腺病毒疣足 (Adenovirus knob, Ad-knob) 基因序 列, 引物分别是: F1 (5'GAAAGCTAGC CCTGCAAACATCA 3')、R1 5'ACTCCCGGGAGTTGTGTCTCCTGTTTCCTG F2 5'ACTCCCGGGAGTGC ATACTCTATGTCA 3'), R2 (5'TATGGTAC CGGGAGGTGGTGA 3'), F3(5'AACCTAGGGAGGTTAACCTAAGCACTG 3'), 和 R3 (5'CTCAAGCTTTTTGG AATTGTTTGA 3')。以引物 F1-R1、F2-R2、 F3-R1 和 F2-R3 分别进行第一轮 PCR, 得到产物 PCR1、PCR2、PCR3 和 PCR4, 再以 F1-R2 和 F3-R3 为引物,以第一次扩增产物 PCR1 与 PCR2、PCR3 与 PCR4 为模板进行第二轮 PCR 扩增,得到 PCR 产物 PCR1-PCR2(PCR I)、PCR3-PCR4 (PCR II),将第二轮 PCR 扩增的 PCR I 和 PCR II 插入到经 EcoRV 酶切的 pBR322 载体中,所得到的重组质粒命名为 pBR-PCR I 和 pBR-PCR II。把 RGD-4C 双螺旋 寡聚核苷酸 (RGD-4C duplex):

5'TGTGACTGCCGCGGAGACTGTTTCTGC 3'

3'ACACTGACGGCGCCTCTGACAAAGACG 5'

插入到 Smal 酶切的 pBR-PCR I and pBR-PCR II 载体中,将所得到的重组质粒命名为 pBR-PCR/RGD I 和 pBR-PCR/RGD II,并对重组结构进行测序确证。用 Nhel/KpnI 双酶切 pBR-PCR/RGD I,电泳回收 PCR/RGD I 片段,然后插入到 Nhel/KpnI 双酶切的 pUC-AdFiber I 载体中,所得的重组质粒命名为 pUC-AdFiber-RGD I;用 AvrII/HindIII 双酶切 pBR-PCR/RGD II,电泳回收 PCR/RGD II 片段,再插入到 AvrII/HindIII 双酶切的 pUC-AdFiber II 载体中,将所得到的重组质粒命名为 pUC-AdFiber-RGD II。然后,用 SpeI/PacI 双酶切 pUC-AdFiber-RGD I 和 pUC-AdFiber-RGD II 载体,电泳回收 AdFiber-RGD I、AdFiber-RGD II 片段,插入到 SpeI/PacI 双酶切的 pAdEasy-1、pAdEasy-2 载体中,所得的重组质粒分别命名为 pAdEasy-RGD I、pAdEasy-RGD II。将经 PmeI 线性化的腺病毒穿梭质粒 pShuttle-hEGFR、pShuttle-mEGFR 和 pShuttle-chEGFR 分别与 pAdEasy-RGD I 和 pAdEasy-RGD II 共转化 E.coli BJ5183,所得的重组质粒命名为腺病毒质粒 pAd-RGD-EGFR I 和 pAd-RGD-EGFR II,腺病毒质粒 pAd-RGD-EGFR I 转染 293 细胞,所得的重组腺病毒命名为 Ad-RGD-EGFR I,腺病毒质粒 pAd-RGD-EGFR II

转染 293E4pIX 细胞,所得的重组腺病毒命名为 Ad-RGD-EGFR Ⅱ。经纯化的 Ad-RGD-EGFR I 和 Ad-RGD-EGFR Ⅱ 可作为疫苗进行免疫,对肿瘤血管内皮细胞 具有特异的靶向性。

本发明发现纳米靶向的 EGFR 分子疫苗能比各种常规的 EGFR 分子疫苗更有效地提高 EGFR 的免疫原性,诱导更强烈的抗肿瘤免疫反应。纳米靶向 EGFR 分子疫苗抗肿瘤作用原理示意图见图 6。

5. EGFR 分子疫苗与其它免疫反应促进因子等的协同应用

EGFR 分子疫苗的抗肿瘤作用效应可在其它免疫促进因子的协同作用下得到增强,这些免疫促进因子包括各种细胞因子(cytokine,如 IL-2、TNF-、IFN-、GM-CSF等)、各种趋化因子(Chemokine,如 MIP3 、MIP3 、IP10等)、各种应急因子(如 HSP70、HSP90等)、各种免疫刺激因子(如 B7等)。各种免疫促进因子与 EGFR 分子疫苗的协同作用既可以通过基因融合在基因水平上实现,也可以通过蛋白质联合应用在蛋白质水平上实现,还可以通过共转染肿瘤细胞、树突状细胞和血管内皮细胞等在细胞水平上实现。

六、 EGFR 分子疫苗的抗肿瘤作用效应

EGFR 分子疫苗的重要生物学功能就是以同种、异种或直接进化的 EGFR 分子为抗原分子所构建的各种新生物技术疫苗,包括蛋白疫苗、基因疫苗、病毒疫苗和基因修饰疫苗等,都具有抗肿瘤作用,这些抗肿瘤作用表现为保护性抗肿瘤作用、治疗性抗肿瘤作用以及抗肿瘤转移作用。

为观察 EGFR 重组 DNA 疫苗的抗肿瘤效应,给随即分组的小鼠(每组 15 只)分别肌肉注射 100 g的 EGFR 重组 DNA 疫苗 hEe-p、mEe-p、c-p 或生理盐水,每周一次,连续四周。在第四次免疫结束后一周,给每只免疫小鼠分别皮下接种5×10⁵ 个 LL/2c Lewis 肺癌(图 7 A 和 C)或 MA782/5S 乳腺癌细胞(图 7 B 和 D)。从图中可以看出,用 mEe-p、c-p 或生理盐水免疫小鼠的肿瘤持续生长,而 hEe-p 免疫小鼠的保护性免疫效应则非常明显,并且 hEe-p 免疫小鼠的生存率也明显高于mEe-p、c-p 或生理盐水免疫小鼠,hEe-p 免疫的小鼠生存期超过 5 个月,其在荷瘤 150 天后,接种 LL/2c Lewis 肺癌和 MA782/5S 乳腺癌的生存率分别有 60%和66%。研究还发现,保护性免疫效应呈剂量依存效应,150 g 剂量的免疫效果与100 g 的免疫效果相当,而 5-15 g 的剂量则几乎没有免疫效果。此外,hEe-p 免疫 EGFR 阴性肿瘤(如 H22 肝癌和 MMT-06052 鼠乳腺癌)的荷瘤小鼠没有保护性免疫效应。

除保护性免疫效应外,EGFR 重组 DNA 疫苗还具有治疗性免疫效应(图 8)。同样地,给随即分组的小鼠(每组 15 只)首先皮下接种 1 10⁶个 LL/2c Lewis 肺癌(图 8 A 和 C)或 MA782/5S 乳腺癌活细胞 (图 8 B 和 D), 5 天后分别肌肉注射 100 g 的 hEe-p、mEe-p、c-p 或生理盐水,每周一次,连续四周。从图中可以看出,用 mEe-p、c-p 或生理盐水免疫小鼠的肿瘤持续生长,而 hEe-p 免疫小鼠的治

疗性免疫效应则非常明显,并且 hEe-p 免疫小鼠的生存率也明显高于 mEe-p、c-p 或生理盐水免疫小鼠,hEe-p 免疫的小鼠生存期超过 5 个月,其在荷瘤 150 天后,接种 LL/2c Lewis 肺癌和 MA782/5S 乳腺癌的生存率分别有 40%和 53%。

重组 EGFR 蛋白疫苗也同样有保护性免疫效应和治疗性免疫效应(图 9)。同前文所述,选择 6-8 周龄雌性 C57BL/6 或 BALB/c 小鼠,随机分组,建立 LL/2c Lewis 肺癌、MA782/5S 乳腺癌和 C26 结肠癌荷瘤小鼠模型。荷瘤小鼠皮下注射重组蛋白疫苗 5-50μg 或佐剂氢氧化铝磷胶及生理盐水 100μl,每周一次,连续四周。重组 chEGFR 蛋白疫苗免疫小鼠产生了明显的抗肿瘤保护性免疫效应和治疗性免疫效应,可抑制 LL/2c Lewis 肺癌和 MA782/5S 乳腺癌的生长,延长荷瘤小鼠的生存时间,但对 EGFR 阴性的 C26 肿瘤无明显的影响。而用重组 mEGFR 蛋白疫苗、铝佐剂免疫或注射生理盐水等对照组的荷瘤小鼠的肿瘤则生长迅速,小鼠生存期明显缩短。疫苗组肿瘤体积(t 检验)和生存期(log-rank 检验)与各对照组比较均有显著差异(P<0.05)。图 9 就显示了重组 EGFR 蛋白疫苗对 MA782/5S 乳腺癌荷瘤小鼠的抗肿瘤作用效果。

转移是肿瘤进展以及放化疗失败的常见原因,肿瘤细胞在血液、淋巴循环中的存在以及微小转移灶的形成是转移的关键。本发明继续观察到重组 EGFR 分子疫苗还有抗肿瘤转移作用 (图 10)。在尾静脉注射转移模型的治疗中发现,chEGFR蛋白疫苗免疫的荷瘤小鼠较少发生肺部转移或程度远较对照组轻,而重组 mEGFR蛋白疫苗、铝佐剂免疫或注射生理盐水等对照组的荷瘤小鼠则 100%出现肺部转移,且转移病灶的个数明显为多,甚至无法计数。图 10显示了 LL/2c Lewis 肺癌荷瘤小鼠经重组 EGFR 蛋白疫苗免疫的抗肿瘤转移效应,从图中可以看出,各组治疗结束时,chEGFR蛋白疫苗免疫小鼠的 LL/2c 移植瘤肺部转移灶数量和肺湿重均显著较对照组为轻。

此外, EGFR 分子疫苗还能抑制体外 (in vitro) 肿瘤细胞的生长及具有体内 (in vivo) 过继免疫抗肿瘤效应 (图 11)。EGFR 分子疫苗第四次免疫 7 天后,收集小鼠血清, 用亲和层析法纯化血清中的免疫球蛋白 (immunoglobulin, Ig)。向 2×10⁵/ml 的处于对数生长期的 EGFR 阳性肿瘤细胞(A549, LL/2c, MA782/5S)和 EGFR 阴性肿瘤细胞(H22 和 MMT-06052)中加入不同浓度的 Ig(1-1000 mg/ml)共培养72 小时, 用台盼蓝染色法鉴定活细胞, 计算细胞生长抑制率。结果显示用人 EGFR 重组 DNA 疫苗 hEe-p 免疫小鼠来源的 Ig 处理的 EGFR 阳性肿瘤细胞呈现明显的抑制生长状态,而 EGFR 阴性的肿瘤细胞则没有影响(图 11A)。作为对照,用未免疫的正常小鼠来源的 Ig 与相应的肿瘤共培养,无论对 EGFR 阳性肿瘤细胞还是EGFR 阴性肿瘤细胞都没有抑制作用(图 11B)。

这些纯化的来源于免疫小鼠的 Ig 还具有过继免疫作用效应。裸鼠皮下接种 $I \times 10^5 - 1 \times 10^6$ 个肿瘤细胞 1 天后,按 10-300 mg/kg 静脉输注纯化的 Ig,每周二次,连续三周。结果显示过继输入的来源于人 EGFR 重组 DNA 疫苗 hEe-p 免疫小鼠的

Ig 具有显著的肿瘤抑制作用。作为对照,把纯化的 Ig 与固定的 EGFR 阳性肿瘤细胞或 EGFR 阴性肿瘤细胞在 4°C下振荡 1 小时以吸附 Ig,共重复 4 次。结果发现来源于人 EGFR 重组 DNA 疫苗 hEe-p 免疫小鼠的 Ig 的抗肿瘤效应由于被 EGFR 阳性肿瘤细胞(LL/2c, MA782/5S)预吸附而消除,但对 EGFR 阴性肿瘤细胞(H22)仍然有效(图 11C)。

除上述过表达 EGFR 的肺癌、乳腺癌外,EGFR 分子疫苗对其他各种过表达 EGFR 的实体瘤,包括卵巢癌、结肠癌、前列腺癌、胃癌、膀胱癌、头颈部鳞癌和胶质瘤等,也有较好的抗肿瘤作用效应。

七、 EGFR 分子疫苗的抗肿瘤作用原理及生物安全性

现代免疫学认为,抗原或抗原表位与相应受体间的相互识别除特异性外,还存在一定程度的伸展性(plasticity)、是混杂性(promiscuity)或退变性(degeneracy),从而通过分子模拟(molecular mimicry)机制诱导针对自身抗原的免疫交叉反应,打破免疫耐受。

本发明发现 EGFR 分子作为一种免疫交叉抗原,能打破机体对自身 EGFR 分子的免疫耐受,诱导机体产生针对 EGFR 分子自身免疫样交叉反应,这些免疫反应包括针对 EGFR 分子的主动免疫反应(包括细胞免疫反应和体液免疫反应)和被动免疫反应(过继免疫反应)。

为了研究 EGFR 分子疫苗的抗肿瘤作用原理,我们首先对 EGFR 分子疫苗免疫小鼠进行体液免疫检测,包括应用流式细胞术、Western 印迹、免疫沉淀等验证小鼠/兔免疫血清中抗 EGFR 自身抗体的存在,应用 ELISA 检测小鼠/兔免疫血清中抗 EGFR 抗体的滴度及抗体类型,应用免疫组织化学法检测免疫小鼠肿瘤组织内的自身抗体,应用肿瘤细胞成集落实验、血清过继免疫实验检测自身抗体的功能等。

每只小鼠在免疫前及免疫后每周经鼠尾静脉采血或处死小鼠时取血,收集血清备用。Western Blot 检测表明重组 EGFR 分子疫苗免疫诱导的抗体可以特异识别相应的免疫原(重组 EGFR 蛋白或表达于肿瘤细胞上的 EGFR),但不能识别 EGFR 阴性细胞。同时,用流式细胞仪对所获得的抗体空间表位进行识别,也发现重组 EGFR 分子疫苗能诱导产生特异的抗体,可以识别表达于肿瘤细胞表面的 EGFR。 ELISA 自身抗体测定结果显示,在初次免疫 2 周后重组 EGFR 分子疫苗免疫小鼠 开始产生抗小鼠自身 EGFR 抗体,滴度可达 1:100 至 1:5000,以后逐渐升高,至第4 周抗体滴度可达 1:10000 至 1:500000,到第8 周抗体滴度仍能维持在 1:500 至 1:1000,而相应的对照组则检测不到明显的抗体产生。对所产生的抗体亚型作进一步检测,发现 EGFR 分子疫苗产生的抗体以 IgG 为主。图 12 显示了 EGFR 分子疫苗(重组蛋白疫苗和重组 DNA 疫苗)免疫小鼠所诱导产生的抗体类型,从图中可以看出,重组 EGFR 分子疫苗明显诱导产生了 IgG1、IgG2a 和 IgG2b等抗体,但 IgM 和 IgA 并没有升高,并且这些抗体可被 CD4 抗体阻断,但不能被抗 CD8、抗

NK 或对照抗体所阻断,而其他对照组蛋白疫苗、佐剂和生理盐水组则不能检测到特异抗体。

EGFR 分子疫苗还诱导了细胞免疫。对 EGFR 分子疫苗免疫的小鼠进行的细胞免疫检测主要包括:应用 51Cr 释放法测定 CTL 活性,应用 ELISPOT 检测细胞因子水平(主要是免疫血清中 IFN-γ和 IL-4 浓度),应用免疫细胞去除 (Depletion of immune cell subsets) 实验检测 T 细胞类型 (CD4+T 淋巴细胞、CD8+T 淋巴细胞或 NK 细胞等)。

重组 chEGFR 疫苗免疫小鼠后,取脾单个核细胞作 ELISPOT 检测,发现脾单 个核细胞中存在大量抗原特异 B 细胞,而经重组 mEGFR 疫苗免疫后也能发现少 量特异 B 细胞的存在,但空白组及佐剂或空载体组未能发现有统计意义的特异 B 细胞。重组 EGFR 分子疫苗免疫小鼠 3 周后,取免疫小鼠脾 T 细胞,并用健康脾 单个核细胞为抗原呈递细胞加以免疫原在体外再刺激扩增可能已活化 T 细胞。结 果显示经重组 chEGFR 分子疫苗免疫后均含有较多量的 IFN- 及 IL-4 产生细胞, 而重组 mEGFR 分子疫苗在体外再刺激源于 chEGFR 疫苗免疫小鼠的脾 T 细胞也 含有较多的 IFN- 及 IL-4 产生细胞, 明显多于同等条件下未体外刺激的 T 细胞。 进一步以标准的 51Cr 释放法测定了重组 EGFR 分子疫苗免疫后脾 T 细胞对鼠、人 等相关肿瘤细胞的特异杀伤活性。结果显示重组 chEGFR 分子疫苗免疫后的小鼠 脾 T 细胞在 40: 1 时对 EGFR 阳性表达 LL/2c、MA781/5S 肿瘤细胞的特异杀伤活 性分别达 40.27%、42.83%,但对重组 mEGFR 分子疫苗免疫的小鼠脾 T 细胞对 MA781/5S 或 LLC 肿瘤细胞、以及 EGFR 阴性 C26 细胞未发现杀伤活性,同时这 种作用可被相应抗 CD8 及 MHC-I 单克隆抗体阻断,而不能被抗 CD4 及 MHC-II 单克隆抗体阻断,其他对照组牌 T 细胞则未检测到有统计意义的杀伤活性。图 13 显示了 EGFR 重组 DNA 疫苗免疫小鼠所表现出的 CTL 效应,从图中可以看出: 人 EGFR 重组 DNA 疫苗 hEe-p 免疫小鼠来源的 T 细胞比其他对照组来源的 T 细胞 对 EGFR 阳性的肿瘤细胞有着更高的细胞毒性,并且这种细胞毒性在体外可被抗 CD8 或抗 MHC I 单抗所阻断, 但不能被抗 CD4 单抗所阻断, 显示这种细胞杀伤活 性来源于 MHC I 依赖的 CD8⁺T 细胞。另外,激活的脾细胞对 YAC-1 靶细胞没有 显示出 NK 活性的增加。此外,过继输注人 EGFR 重组 DNA 疫苗 hEe-p 免疫小鼠 来源的 CD4 剔除 (CD8+) 或 CD8 剔除 (CD4+) T 细胞显示出对 EGFR 阳性肿瘤 细胞的抗肿瘤作用,但对同源的 EGFR 阴性肿瘤细胞的抗肿瘤作用不明显,而相 应的对照组也未显示出抗肿瘤作用效果。

本发明还对 EGFR 分子疫苗免疫小鼠的潜在长期毒性进行了观察。研究中没有观察到诸如小鼠体重减轻、皮毛皱缩、食欲减退、寿命缩短等明显的毒副作用。通过对免疫小鼠的肝、肺、脾、脑等器官的显微病理检查,没有发现病理变化,并且免疫荧光染色也未在主要脏器中发现自身抗体沉着(图 14)。

附图说明

- 图 1 同源分子疫苗的作用原理示意图
- 图 2 重组 EGFR 质粒图谱 A. 重组 EGFR 真核表达质粒图谱; B.重组 EGFR 原核表达质粒图谱; C. 重组 EGFR 酵母表达质粒图谱; D.EGFR 重组腺病毒穿梭质粒图谱; E. EGFR 重组 Lentivirus 病毒前体质粒图谱
 - 图 3 EGFR 重组蛋白疫苗的构建流程示意图
- 图 4 EGFR 重组病毒疫苗的构建流程示意图 A. EGFR 重组腺病毒疫苗的构建流程示意图; B. EGFR 重组 Lentivirus 病毒疫苗的构建流程示意图
 - 图 5 RGD 修饰的腺病毒重组 EGFR 疫苗构建流程示意图
 - 图 6 纳米靶向 EGFR 分子疫苗抗肿瘤作用原理示意图
- 图 7. 重组 EGFR DNA 疫苗的抗肿瘤保护性免疫效应 A. LL/2c Lewis 肺癌荷瘤的免疫小鼠的肿瘤体积变化; B. MA782/5S 乳腺癌荷瘤的免疫小鼠的肿瘤体积变化; C. LL/2c Lewis 肺癌荷瘤的免疫小鼠的生存率; D. MA782/5S 乳腺癌荷瘤的免疫小鼠的生存率。hEe-p,人 EGFR 胞外段 DNA 疫苗; mEe-p,鼠 EGFR 胞外段 DNA 疫苗; c-p,空质粒对照; Saline,生理盐水对照。
- 图 8. 重组 EGFR DNA 疫苗的抗肿瘤治疗性免疫效应 A. LL/2c Lewis 肺癌荷瘤的免疫小鼠的肿瘤体积变化; B. MA782/5S 乳腺癌荷瘤的免疫小鼠的肿瘤体积变化; C. LL/2c Lewis 肺癌荷瘤的免疫小鼠的生存率; D. MA782/5S 乳腺癌荷瘤的免疫小鼠的生存率。 hEe-p,人 EGFR 胞外段 DNA 疫苗; mEe-p,鼠 EGFR 胞外段 DNA 疫苗; c-p,空质粒对照。

图 9 重组 EGFR 蛋白疫苗的抗肿瘤免疫效应 A. 保护性免疫效应; B. 治疗性免疫效应; C. 荷瘤(MA782/5S 乳腺癌)小鼠生存曲线。edCER, 鸡 EGFR 胞外段重组蛋白疫苗; edMER, 鼠 EGFR 胞外段重组蛋白疫苗; Adj, 铝佐剂; NS, 生理盐水。

图 10 重组 EGFR 蛋白疫苗的抗肿瘤转移效应, A. LL/2c 肿瘤肺部转移灶数量; B. LL/2c 肿瘤转移后肺湿重。edCER, 鸡 EGFR 胞外段重组蛋白疫苗; edMER, 鼠 EGFR 胞外段重组蛋白疫苗; Adj, 铝佐剂; NS, 生理盐水。

图 11 EGFR 分子疫苗的体外 (in vitro) 抑制肿瘤细胞生长效应及体内 (in vivo) 过继免疫抗肿瘤效应 A. EGFR 重组 DNA 疫苗 hEe-p 免疫小鼠来源的 Ig 对 EGFR 阳性肿瘤细胞(A549, LL/2c, MA782/5S)和 EGFR 阴性肿瘤细胞(H22 and MMT-06052)的生长抑制状况。B.用未免疫的正常小鼠来源的 Ig 对肿瘤细胞的生长抑制状况。C. EGFR 重组 DNA 疫苗 hEe-p 免疫小鼠来源的 Ig 的体内 (in vivo) 过继免疫抗肿瘤效应。hEe-p,人 EGFR 胞外段 DNA 疫苗;mEe-p,鼠 EGFR 胞外段 DNA 疫苗;c-p,空质粒对照;Saline,生理盐水对照。

图 12 EGFR 分子疫苗诱导产生的抗体类型 A. EGFR 重组 DNA 疫苗诱导产生的抗体类型; B. EGFR 重组蛋白疫苗诱导产生的抗体类型。hEe-p, 人 EGFR 胞

外段 DNA 疫苗; mEe-p, 鼠 EGFR 胞外段 DNA 疫苗; c-p, 空质粒对照; Saline, 生理盐水对照; edCER, 鸡 EGFR 胞外段重组蛋白疫苗; edMER, 鼠 EGFR 胞外段重组蛋白疫苗; Adi, 铝佐剂; NS, 生理盐水。

图 13 EGFR 重组 DNA 疫苗免疫小鼠的 CTL 效应 A. 人 EGFR 重组 DNA 疫苗 hEe-p 及其对照组免疫小鼠来源的 T细胞对 LL/2c Lewis 肺癌细胞在不同 E:T 比例下的 ⁵¹Cr 释放分析结果; B. 过继输注 2×10⁷个来源于人 EGFR 重组 DNA 疫苗 hEe-p 免疫小鼠的 CD4 剔除 (CD8⁺) T细胞对 EGFR 阳性的 LL/2c Lewis 肺癌细胞和 EGFR 阴性的 B16 黑色素瘤细胞的抗肿瘤作用; C. 过继输注 2×10⁷个来源于人 EGFR 重组 DNA 疫苗 hEe-p 免疫小鼠的 CD8 剔除 (CD4⁺) T细胞对 EGFR 阳性的 LL/2c Lewis 肺癌细胞和 EGFR 阴性的 B16 黑色素瘤细胞的的抗肿瘤作用; D. 过继输注 2×10⁷个来源于人 EGFR 重组 DNA 疫苗 hEe-p 免疫小鼠的 CD4 剔除 (CD8⁺) T细胞对 EGFR 阳性的 MA782/5S 乳腺癌细胞和 EGFR 阴性的 MethA 纤维肉瘤细胞的的抗肿瘤作用; E. 过继输注 2×10⁷个来源于人 EGFR 重组 DNA 疫苗 hEe-p 免疫小鼠的 CD8 剔除 (CD4⁺) T细胞对 EGFR 阳性的 MA782/5S 乳腺癌细胞和 EGFR 阴性的 MethA 纤维肉瘤细胞的抗肿瘤作用。□,人 EGFR 重组 DNA 疫苗 hEe-p 免疫小鼠来源的 T细胞过继免疫的荷瘤小鼠; ■,非免疫小鼠来源的 T细胞过继免疫的荷瘤小鼠; ■,非免疫小鼠来源的 T细胞过继免疫的荷瘤小鼠; ■,非免疫小鼠来源的 T细胞过继免疫的荷瘤小鼠;

图 14 肿瘤细胞及组织自身抗体的免疫荧光显微图谱 A. EGFR 分子疫苗免疫的 LL/2c Lewis 肺癌细胞的自身抗体沉着; B. EGFR 分子疫苗免疫的 MA782/5S 鼠乳腺癌细胞的自身抗体沉着; C.未免疫的 LL/2c Lewis 肺癌细胞无自身抗体沉着; D.未免疫的 MA782/5S 鼠乳腺癌细胞无自身抗体沉着; E. CD4⁺T 阻断的 EGFR 分子疫苗免疫的 LL/2c Lewis 肺癌细胞无自身抗体沉着; F. CD4⁺T 阻断的 EGFR 分子疫苗免疫的 MA782/5S 鼠乳腺癌细胞无自身抗体沉着; F. CD4⁺T 阻断的 EGFR 分子疫苗免疫的 MA782/5S 鼠乳腺癌细胞无自身抗体沉着; G. CD8⁺T 阻断的 EGFR 分子疫苗免疫的 LL/2c Lewis 肺癌细胞显示自身抗体沉着; H. CD8⁺T 阻断的 EGFR 分子疫苗免疫的 MA782/5S 鼠乳腺癌细胞显示自身抗体沉着; I. EGFR 分子疫苗免疫小鼠的肝脏无自身抗体沉着; J. EGFR 分子疫苗免疫小鼠的肾脏无自身抗体沉着。

具体实施方式

实施例 1 EGFR 重组 DNA 疫苗

依据 GenBank 等各公开的数据库所收藏的人、小鼠、鸡、的 EGFR 分子的 cDNA 序列(分别对应于 SEQ ID NO 1-5,7-9,19)设计 PCR 引物(人的引物为: 5'GACCATG GAGGAAAAGAAAGTTTGC 3', 5'ACGAATTCTTAGGACGGGATCTTAGGCCCA 3'; 小鼠的引物为: 5'GACCATGGAGGAAAAGAAAGTCTGC 3', 5'ACGAATTC TTAATAGATGGTATCTTTGGC 3'; 鸡的引物为: 5'GACCATGGAGGAGAAGAA

AGTTTGTC 3', 5'ACGAATTCTTAAGATGGAGTTTTGGAGCC 3'), 分别以人肺癌细胞株 A431、小鼠肺癌细胞株 LL2 和鸡胚的总 RNA 为模板进行 RT-PCR 扩增,电泳收集纯化扩增的 EGFR 片段(均为 1.9kb), 然后进行 PCR 产物亚克隆。把 PCR 亚克隆测序确证后,用 NcoI 和 EcoRI 酶切, 收集 1.9kb 片段并纯化, 插入到用 NcoI 和 EcoRI 双酶切的 pORF-MCS(InvivoGen 公司)载体,筛选重组质粒。候选的重组质粒经限制性酶切分析及 PCR 扩增等双重鉴定,命名为 pORF-hEGFR、pORF-mEGFR 和 pORF-chEGFR。

同样地,设计针对于真核表达载体 pcDNA3.1(+)(Invitrogen 公司)的 PCR 引物,分别是:人的引物 5'GAGCTAGCATGGAGGAAA AGAAAGTTTGC 3',5'CACTCGAGTTAGGACGGGATCTTAGGCCCA 3';小鼠的引物为:5'GAGCTAGCATGGAGGAAAAGAAAGTCTGC 3',5'CACTCGAG TTAATAGATGGTATCTTTGGC 3';鸡的引物为:5'GAGCTAGCATGGAGGAGAA GAAAGTTTGTC 3',5'CACTCG AGTTAAGATGGAGTTTTTGGAGCC 3',依前述 RNA 模板进行 RT-PCR扩增。将纯化的 1.9kb 的 PCR 产物用 NheI 和 XhoI 双酶切,插入到对应的双酶切载体 pcDNA3.1(+)中,筛选重组质粒,将重组质粒分别命名为 pcDNA-hEGFR、pcDNA-mEGFR 和 pcDNA-chEGFR。

这些真核表达质粒 pORF-EGFR 或 pcDNA-EGFR 等转染入 CHO、NIH3T3、Vero 等细胞系,观察并用 SDS-PAGE、ELISA、Western Blot 等技术检测其表达 EGFR 的情况和水平。用碱法抽提经过鉴定的重组 EGFR 分子表达质粒,用超离心、超滤等方法去除大肠杆菌内毒素,即得到纯净的质重组粒 DNA,这些质粒 DNA 即可作为 DNA 疫苗进行免疫。

概括地讲,EGFR 重组 DNA 疫苗的构建过程是: 依据 GenBank 等各公开的数据库所收藏的各种生物来源(如人、小鼠、大鼠、鸡、鲐、果蝇等,见 SEQ ID NO 1-14)的关于 EGFR 分子的序列(包括基因、cDNA、mRNA 和氨基酸序列)用常规方法设计引物或探针,用 PCR、RT-PCR、杂交等技术,从各种商品化的基因文库、cDNA 文库(ClonTech, Strategene 公司等生产的各种基因文库、cDNA 文库)或各种细胞系(如人肺癌细胞株 A431、鼠 Lewis 肺癌细胞株 LL2等)、组织(如肺癌、乳腺癌组织、果蝇、鸡胚等)等中克隆分离出不同种属生物来源的 EGFR分子的胞外区段的 cDNA,或进一步用基因直接进化技术筛选到的具有强免疫原性的 EGFR分子。各种来源的 EGFR cDNA 的胞外段序列经测序鉴定后,用分子克隆技术插入真核生物表达质粒(如 pcDNA3.1、pORF-mcs、pBLAST-mcs、pSecTag2等),筛选重组表达质粒(经限制性酶切分析鉴定后转染入 CHO、NIH3T3、Vero等细胞系,观察并用 SDS-PAGE、ELISA、Western Blot等技术检测其表达 EGFR的情况和水平。用碱法抽提经过鉴定的重组 EGFR 分子表达质粒,用超离心、超滤等方法去除大肠杆菌内毒素,即得到纯净的质重组粒 DNA,这些质粒 DNA 即可作为 DNA 疫苗进行免疫。

选择 6-8 周龄小鼠,用常规方法建立各种荷瘤小鼠模型,每只小鼠注射 100 g EGFR 重组表达质粒,每周 1 次,连续 4 周。观察荷瘤小鼠的生长情况及肿瘤的发展情况,8 周后处死小鼠,收集免疫小鼠血清及各种脏器,用流式细胞术、ELISA、Western blot 等方法检测体液免疫反应,用 Cr⁵¹、ELISpot 等检测细胞免疫反应,用免疫组化方法检测免疫的毒、副作用。进一步提纯免疫小鼠血清,按常规方法建立裸鼠的肿瘤模型,进行过继免疫治疗,观察肿瘤的生长情况。

实施例 2 EGFR 重组蛋白疫苗 (E.coli 表达)

同前文所述, 依据 GenBank 等各公开的数据库所收藏的人、小鼠、鸡、的 EGFR 分子的 cDNA 序列(分别对应于 SEQ ID NO 1-5,7-9,19)设计 PCR 引物(人的引物为: 5'GACCATGGAGGAAAAGAAAGTTTGC 3', 5'ACAGATCTAGGACGGGA TCTTAGGCCCA 3'; 小鼠的引物为: 5'GACCATGGAGGAAAAGAAAGTCTGC 3', 5'ACAGATCTATAGATGGTATCTTTGGC 3'; 鸡的引物为: 5'GACCATGGA GGA GAAGAAAGTTTGTC 3', 5'ACAGATCTATAGATGGTATCTTTGGC 3'; 鸡的引物为: 5'GACCATGGA GGA GAAGAAAGTTTGTC 3', 5'ACAGATCTAGATGGAGTTTTGGAGCC 3'), 分别以 pORF-hEGFR、pORF-mEGFR 和 pORF-chEGFR 等为模板进行 PCR 扩增,电泳收集纯化扩增的 EGFR 片段(均为 1.9kb), 然后进行 PCR 产物亚克隆。把 PCR 亚克隆测序确证后,用 NcoI 和 BgIII 酶切,收集 1.9kb 片段并纯化,插入到用 NcoI 和 BgIII 双酶切的 pQE60(QIAGEN 公司)载体,筛选重组质粒。候选的重组质粒经限制性酶切分析及 PCR 扩增等双重鉴定,命名为 pQE-hEGFR、pQE-mEGFR 和 pQE-chEGFR。

同样地,可针对其他不同的原核表达载体(如 pET32、pLLp、pSE420 等)设计 PCR 引物,以构建其他的重组原核表达质粒。

经限制性酶切分析等鉴定后的各重组原核表达质粒,转化不同的大肠杆菌宿主(如 E.coli TOP10F'、E.coli BL21(DE3)pLys、E.coli M15、E.coli DH5 、 E.coliJM109等),观察并用 SDS-PAGE、ELISA、Western Blot 等技术检测其表达 EGFR 的情况和水平,确定最适的大肠杆菌表达宿主菌株。用经确证的重组 EGFR 表达质粒转化最适大肠杆菌菌株,建立稳定表达菌株和各级种子库。重组菌株大量摇瓶培养或发酵,低温离心收集菌体,菌体经 PBS 重悬后用超声法破碎细胞,用离子交换层析、亲和层析等方法分离纯化重组 EGFR 蛋白,此重组的 EGFR 蛋白质即可作为蛋白疫苗进行免疫。

选择 6-8 周龄小鼠,用常规方法建立各种荷瘤小鼠模型,每只小鼠注射 5-50 g EGFR 重组蛋白,每周 1 次,连续 4 周。观察荷瘤小鼠的生长情况及肿瘤的发展情况,8 周后处死小鼠,收集免疫小鼠血清及各种脏器,用流式细胞术、ELISA、Western blot 等方法检测体液免疫反应,用 Cr⁵¹、ELISpot 等检测细胞免疫反应,用免疫组化方法检测免疫的毒、副作用。进一步提纯免疫小鼠血清,按常规方法建立裸鼠的肿瘤模型,进行过继免疫治疗,观察肿瘤的生长情况。

实施例3 EGFR重组蛋白疫苗(酵母Pichia pastoris表达)

同前文所述,依据 GenBank 等各公开的数据库所收藏的人、小鼠、鸡、的 EGFR 分子的 cDNA 序列(分别对应于 SEQ ID NO 1-5,7-9,19)设计 PCR 引物(人的引 物为: 5'ATACTCGAGAAAAGAGAGGGAAAAGAAAG 3', 5' GCTCTA GAATGGCACAGGTGGCACA 3'; 小鼠的引物为: 5'ATGCTCGAGAAAA GAGAG TTGGAGGAAAAGAAAGTC 3', 5'AAGCGGCCGCCATAGATGGTATCTTTG 3'; 鸡的引物为: 5'ATACTCGAGAAAAGAGAGGTGGAGGAGAAGAAG 3', 5'C GTCTAGAAGATGGAGTTTTGGAG 3'), 分别以 pORF-hEGFR、pORF-mEGFR 和 pORF-chEGFR 等为模板进行 PCR 扩增,电泳收集纯化扩增的 EGFR 片段(均为 1.9kb),然后进行 PCR 产物亚克隆。把 PCR 亚克隆测序确证后,用 XhoI 和 XbaI(对 小鼠的克隆为 XhoI 和 NotI)酶切,收集 1.9kb 片段并纯化,插入到用 XhoI 和 XbaI 双酶切(对小鼠的克隆为 XhoI 和 NotI 双酶切)的 pPICZ A (Invitrogen 公司) 载体,转化 E.coli 筛选重组质粒。候选的重组质粒经限制性酶切分析及 PCR 扩增 等双重鉴定,命名为酵母表达质粒 pYE-hEGFR、pYE-mEGFR 和 pYE-chEGFR。 这些酵母表达质粒经 PmeI 酶切线性化后,用电穿孔法转化酵母菌株 X33 或 GS115, 用 Zeocin 抗性筛选稳定转化子,在 MMH (Minimal Methanol with histidine, MMH) 和 MDH(Minimal Dextrose with histidine, MDH)琼脂平板上鉴定和挑选 Mut⁺转 化子。挑选 6-10 个 Mut⁺转化子进行小规模表达,用 SDS-PAGE、Western Blot、ELISA 等方法对所表达的重组蛋白进行鉴定。选择其中表达效率最高的 Mut⁺转化子做大 规模表达,建立各级酵母表达种子库。重组菌株大量摇瓶培养或发酵,低温离心 收集菌体,菌体经 PBS 重悬后用超声法破碎细胞,用离子交换层析、亲和层析等 方法分离纯化重组 EGFR 蛋白,此重组的 EGFR 蛋白质即可作为蛋白疫苗进行免 疫。

同样地,也可用其他的酵母表达系统构建类似的酵母重组表达质粒。

选择 6-8 周龄小鼠,用常规方法建立各种荷瘤小鼠模型,每只小鼠注射 5-50 g EGFR 重组蛋白,每周 1 次,连续 4 周。观察荷瘤小鼠的生长情况及肿瘤的发展情况,8 周后处死小鼠,收集免疫小鼠血清及各种脏器,用流式细胞术、ELISA、Western blot 等方法检测体液免疫反应,用 Cr⁵¹、ELISpot 等检测细胞免疫反应,用免疫组化方法检测免疫的毒、副作用。进一步提纯免疫小鼠血清,按常规方法建立裸鼠的肿瘤模型,进行过继免疫治疗,观察肿瘤的生长情况。

实施例 4 EGFR 重组腺病毒疫苗

同前文所述,依据 GenBank 等各公开的数据库所收藏的人、小鼠、鸡、的 EGFR 分子的 cDNA 序列(分别对应于 SEQ ID NO 1-5,7-9,19)设计 PCR 引物(人的引物为: 5'GAAGATCTATGGAGGAAAAGAAAGTTTGC 3', 5'ACGATATCTTAA GGACGGGATCTTAGGCCCA 3'; 小鼠的引物为: 5'GAAGATCTATGGAGGAAA AGAAAGTCTGC 3', 5'ACGATATCTTAATAGATGGTATCTTTGGC 3', 5'ACGATATCTTA

AGATGGAGTTTTGGAGCC 3'), 分别以 pORF-hEGFR 、pORF-mEGFR 和 pORF-chEGFR 等为模板进行 PCR 扩增,电泳收集纯化扩增的 EGFR 片段(均为 1.9kb), 然后进行 PCR 产物亚克隆。把 PCR 亚克隆测序确证后, 用 BglII 和 EcoRV 酶切,收集 1.9kb 片段并纯化,插入到用 BglII 和 EcoRV 双酶切的 pShuttle-CMV (Quantum Biotechnologies 公司) 载体,筛选重组质粒。候选的重组质粒经限制性 酶切分析及 PCR 扩增等双重鉴定,命名为腺病毒穿梭表达质粒 pShuttle-hEGFR、 pShuttle-mEGFR 和 pShuttle-chEGFR (图 2D)。分别将经 PmeI 酶切的腺病毒穿梭 表达载体 pShuttle-EGFR 与包含腺病毒基因组的骨架载体 pAdEasy-1 或 pAdEasy-2 共转化 E.coli BJ5183, 得到重组腺病毒载体质粒 pAd-hEGFR、pAd-mEGFR 和 pAd-chEGFR。这些重组腺病毒载体质粒经 PacI 酶切后,用磷酸钙-DNA 共沉淀法 转染入腺病毒包装细胞株 293 细胞中,得到相应的重组腺病毒 Ad-hEGFR、 Ad-mEGFR 和 Ad-chEGFR。用 PCR、Western blot 和酶切分析确证 EGFR 基因构 建入重组腺病毒载体中并能在真核细胞中得到正确有效的表达,用超离心法大量 制备重组腺病毒 Ad-EGFR, 用上层琼脂法、TCID50 等法测定每批次的重组腺病 毒的滴度(pfu)。利用 293 细胞大量扩增经确证的 EGFR 重组腺病毒疫苗,用超离 心、超滤等技术分离、纯化重组腺病毒,此经纯化的 EGFR 重组腺病毒即可作为 疫苗进行免疫。

同前文所述,选择 6-8 周龄小鼠,用常规方法建立各种荷瘤小鼠模型,每只小鼠注射 1×109 PFU 的 EGFR 重组腺病毒,每周 1 次,连续 4 周。观察荷瘤小鼠的生长情况及肿瘤的发展情况,8 周后处死小鼠,收集免疫小鼠血清及各种脏器,用流式细胞术、ELISA、Western blot 等方法检测体液免疫反应,用 Cr51、ELISpot等检测细胞免疫反应,用免疫组化方法检测免疫的毒、副作用。进一步提纯免疫小鼠血清,按常规方法建立裸鼠的肿瘤模型,进行过继免疫治疗,观察肿瘤的生长情况。

实施例 5 EGFR 重组 Lentivirus 病毒疫苗

如前文所述,本发明利用 ViraPowerTM Lentiviral Gateway[®] Expression Kit (Invitrogen公司)构建 EGFR 重组 Lentivirus病毒,其具体构建过程是: 依据 GenBank 等各公开的数据库所收藏的人、小鼠、鸡、的 EGFR 分子的 cDNA 序列 (分别对应于 SEQ ID NO 1-5,7-9,19) 设计 PCR 引物 (人的引物为: 5'GACCATGGA GGAAAAGAAAGTTTGC 3', 5'ACGATATCAGGACGGGA TCTTAGGCCCA 3'; 小鼠的引物为: 5'GACCATGGAGGAAAAGAAAGTCTGC 3', 5'ACGATATCA TAGATGGTATCTTTGGC 3'; 鸡的引物为: 5'GACCATGGAGGAGAAAGT TTGTC 3', 5'ACGATATCAGATGGAGTTTTGGAGCC 3'), 分别以 pORF-hEGFR、pORF-mEGFR 和 pORF-chEGFR 等为模板进行 PCR 扩增,电泳收集纯化扩增的 EGFR 片段 (均为 1.9kb),然后进行 PCR 产物亚克隆。把 PCR 亚克隆测序确证后,用 NcoI 和 EcoRV 酶切,收集 1.9kb 片段并纯化,插入到用 NcoI 和 EcoRV 双酶切

的 pENTR11(Invitrogen 公司)载体中,筛选重组质粒,命名为 pENTR-hEGFR、pENTR-mEGFR 和 pENTR-chEGFR(图 2E)。分别将这些载体 pENTR-EGFR 与包含 Lentivirus 病毒基因组的骨架载体 pLenti6/V5-DEST 共转化 E.coli DH5 ,得到重组 Lentivirus 病毒载体质粒 pLenti-hEGFR、pLenti-mEGFR 和 pLenti-chEGFR(图 2E)。这些重组 Lentivirus 病毒载体质粒与包装混合物一起(ViraPower Packaging Mix)用磷酸钙-DNA 共沉淀法转染入 Lentivirus 病毒包装细胞株 293FT 细胞中,得到相应的重组 Lentivirus 病毒 Lenti-hEGFR、Lenti-mEGFR 和 Lenti-chEGFR。用PCR、Western blot 等确证 EGFR 基因构建入重组 Lentivirus 病毒载体中并能在真核细胞中得到正确有效的表达。利用 293FT 细胞大量扩增经确证的 EGFR 重组 Lentivirus 病毒疫苗,用超离心、超滤等技术分离、纯化重组 Lentivirus 病毒,此经纯化的 EGFR 重组 Lentivirus 病毒即可作为疫苗进行免疫。

实施例 6 甘露糖化腺病毒重组 EGFR 疫苗

如前文所述,用常规方法扩增 EGFR 重组腺病毒(I 代、II 代均可),层析或超离心法纯化重组腺病毒。将 70mg mannan(sigma)溶于 5ml 0.1M 的磷酸盐缓冲液(pH6.0)中,终浓度 14mg/ml,加 45ml 0.01M 高碘酸钠溶液,在 4℃下混合氧化 60 分钟,加入 10 1乙二醇,在 4℃下孵育 30 分钟,即得 Ox-M(Oxidative Mannan)混合物。将 Ox-M 混合物倒入用重碳酸盐缓冲液(pH6.0-9.0)平衡 Sephadex-G25层析柱进行层析分离,OX-M 即被洗入 2ml 的空容器。将纯化的 Ox-M 与 1×10¹⁴腺病毒颗粒混合,室温过夜,即获得所需 Ox-M-腺病毒。在 Ox-M-腺病毒中加入1mg/ml 硼氢化钠,室温放置 3 小时即得 Red-M-腺病毒。Ox-M-腺病毒与 Red-M-腺病毒经超滤脱盐、浓缩后,过滤细菌,小管分装,一80℃低温保存。此经纯化的甘露糖化重组 EGFR 腺病毒即可作为疫苗进行免疫。

同前文所述,选择 6-8 周龄小鼠,用常规方法建立各种荷瘤小鼠模型,每只小鼠注射 1×10¹⁰ PFU 的重组 EGFR 甘露糖化腺病毒疫苗,每周 1 次,连续 4 周。观察荷瘤小鼠的生长情况及肿瘤的发展情况,8 周后处死小鼠,收集免疫小鼠血清及各种脏器,用流式细胞术、ELISA、Western blot 等方法检测体液免疫反应,用 Cr⁵¹、ELISpot 等检测细胞免疫反应,用免疫组化方法检测免疫的毒、副作用。进一步提纯免疫小鼠血清,按常规方法建立裸鼠的肿瘤模型,进行过继免疫治疗,观察肿瘤的生长情况。

实施例 7 RGD 修饰的腺病毒重组 EGFR 疫苗的构建

如前文所述,本发明利用 AdEasy 系统构建 RGD 修饰腺病毒的重组 EGFR 疫苗,其具体过程是: 腺病毒基因组骨架质粒 pAdEasy-1 和 pAdEasy-2 经限制性内切酶 SpeI (Sp) 酶切后,用 T4 DNA 聚合酶补平 (filling, f) 末端,再用 PacI (P) 酶切,分别电泳回收 6211bp 和 3579bp 的片段,分别命名为 AdFiber I/Sp/f/P 和 AdFiber II/Sp/f/P,该片段包含了完整的腺病毒纤毛(Adenovirus fiber)基因。把 AdFiber I/Sp/f/P 和 AdFiber II/Sp/f/P 片段插入经 BamHI 酶切一T4 DNA 聚合酶补

平-Pacl 酶切处理(BamHI/filling /Pacl-digested)的 pShuttle 载体,将所得的重组 质粒分别命名为 pSh-AdFiber I 和 pSh-AdFiber II。pSh-AdFiber I 用 NheI 酶切一T4 DNA 聚合酶补平-KpnI 酶切处理 (NheI/filling/KpnI), 电泳回收 2090 bp 的片段 AdFiber I/Nh/f/K,将该片段插入到经 SmaI/KpnI 双酶切的 pUC18 载体中,所得的 重组质粒命名为 pUC-AdFiber I;而 pSh-AdFiber II 用 AvrII 酶切一T4 DNA 聚合酶 补平一HindIII 酶切处理(AvrII/filling/HindIII),电泳回收 838 bp 的片段 AdFiber I/A/f/H,将该片段插入到经 Smal/HindIII 双酶切的 pUC18 载体中,所得的重组质 粒命名为 pUC-AdFiber II。设计一系列 PCR 引物以便以 pUC-AdFiber I 和 pUC-AdFiber II 为模板扩增腺病毒疣足(Adenovirus knob, Ad-knob) 基因序列, 引物分别是: F1(5'GAAAGCTAGC CCTGCAAACATCA 3')、R1(5'ACTCCC GGGAGTTGTCTCCTGTTTCCTG 3')、F2 (5'ACTCCCGGGAGTGCATACTC TATGTCA 3')、R2 (5'TATGGTAC CGGGAGGTGGTGA 3')、F3 (5'AACCTAG GGAGGTTAACCTAAGCACTG 3')、和 R3 (5'CTCAAGCTTTTTGGAATTGT TTGA 3')。以引物 F1-R1、F2-R2 、 F3-R1 和 F2-R3 分别进行第一轮 PCR, 得 到产物 PCR1、PCR2、PCR3 和 PCR4, 再以 F1-R2 和 F3-R3 为引物, 以第一次扩 增产物 PCR1 与 PCR2、PCR3 与 PCR4 为模板进行第二轮 PCR 扩增,得到 PCR 产 物 PCR1-PCR2 (PCR I)、PCR3-PCR4 (PCR II),将第二轮 PCR 扩增的 PCR I 和 PCR II 插入到经 EcoRV 酶切的 pBR322 载体中,所得到的重组质粒命名为 pBR-PCR I 和 pBR-PCR II。把 RGD-4C 双螺旋寡聚核苷酸 (RGD-4C duplex):

5'TGTGACTGCCGCGGAGACTGTTTCTGC 3'

3'ACACTGACGGCGCCTCTGACAAAGACG 5'

插入到 SmaI 酶切的 pBR-PCR I and pBR-PCR II 载体中,将所得到的重组质粒命名为 pBR-PCR/RGD I 和 pBR-PCR/RGD II,并对重组结构进行测序确证。用 NheI/KpnI 双酶切 pBR-PCR/RGD I,电泳回收 PCR/RGD I 片段,然后插入到 NheI/KpnI 双酶切 的 pUC-AdFiber I 载体中,所得的重组质粒命名为 pUC-AdFiber-RGD I;用 AvrII/HindIII 双酶切 pBR-PCR/RGD II,电泳回收 PCR/RGD II 片段,再插入到 AvrII/HindIII 双酶切的 pUC-AdFiber II 载体中,将所得到的重组质粒命名为 pUC-AdFiber-RGD II。然后,用 SpeI/PacI 双酶切 pUC-AdFiber-RGD I 和 pUC-AdFiber -RGD II 载体,电泳回收 AdFiber-RGD I、AdFiber -RGD II 片段,插入到 SpeI/PacI 双酶切的 pAdEasy-1、pAdEasy-2 载体中,所得的重组质粒分别命名为 pAdEasy-RGD I、pAdEasy-RGD II。将经 PmeI 线性化的腺病毒穿梭质粒 pShuttle-hEGFR、pShuttle-mEGFR 和 pShuttle-chEGFR 分别与 pAdEasy-RGD I 和 pAdEasy-RGD II 共转化 E.coli BJ5183,所得的重组质粒命名为腺病毒质粒 pAd-RGD-EGFR I 和 pAd-RGD-EGFR II,腺病毒质粒 pAd-RGD-EGFR I 转染 293 细胞,所得的重组腺病毒命名为 Ad-RGD-EGFR I,腺病毒质粒 pAd-RGD-EGFR II 转染 293E4pIX 细胞,所得的重组腺病毒命名为 Ad-RGD-EGFR II。

经纯化的 Ad-RGD-EGFR I 和 Ad-RGD-EGFR II 可作为疫苗进行免疫, 对肿瘤血管内皮细胞具有特异的靶向性。

实施例 8 重组 EGFR 分子疫苗的药效学观察

药效学研究是观察和验证重组 EGFR 分子疫苗的抗肿瘤作用的重要指标。在本发明中,药效学观察的实验包括:普通荷瘤小鼠(含保护性免疫实验、治疗性免疫实验、肿瘤转移模型实验)、兔免疫试验、猴免疫试验、裸鼠过继免疫实验、剂量依存实验、体外细胞实验等。这里主要叙述以肺癌为模型的普通荷瘤小鼠实验,其它可类比。

- 1. 荷瘤小鼠保护性免疫实验 随机分组的 6-8 周龄 C57BL/6 小鼠双侧后肢股四头肌肌肉交叉注射 EGFR 分子疫苗,每周一次,连续四周。第 5 周各组小鼠均右腋皮下接种 LL/2 肿瘤细胞 1×10^6 。分别在首次免疫第 0、1、3、5 周经鼠尾静脉或处死小鼠时采血。离心后(5000 转/分,3 分钟)收集血清,-20 C保存备用。
- 2. 荷瘤小鼠治疗性免疫实验 随机分组的 6-8 周龄 C57BL / 6 小鼠均右腋皮下接种 LL/2 肿瘤细胞 1×10⁶。接种后第 4 天(可触及肿瘤),随机分组,小鼠后肢肌肉交叉注射。每周一次,连续四周。各组分别在首次免疫第 0、2、4、6 周经鼠尾静脉或处死小鼠时采血。离心后(5000 转/分,3 分钟)收集血清,-20℃保存备用。 观察并记录肿瘤重量、体积及生存曲线。
- 3. 荷瘤小鼠转移模型实验 选 6-8 周龄 C57BL / 6 雌性小鼠,随机分组,小鼠后肢肌肉交叉注射疫苗及对照组。每周一次,连续四周。2 周后,取对数期生长的 LL/2 细胞,按 2×10⁵ /只接种于 C57BL/6 小鼠右后腿肌肉内,继续上述疫苗注射,4 周后断颈处死小鼠,取肺称重,检查并计数肺部转移灶后,用 10%中性缓冲福尔马林固定。

权利要求

- 1. 一种制备疫苗的方法,该方法包括:
- 1) 分析特定病原体的特异抗原;
- 2) 获得编码特异抗原的多核苷酸序列;
- 3) 获得与该多核苷酸序列有足够差异的多核苷酸序列;
- 4) 利用步骤 3) 中所得的多核苷酸序列制备疫苗。
- 2. 如权利要求1所述的方法,其中所述病原体选自细菌、病毒、真菌和原生动物。
- 3. 如权利要求 1 所述的方法,其中步骤 1) 所述的多核苷酸序列与步骤 3) 所述的多核苷酸序列的差异,以它们所编码的多肽序列而言,同源性为 30%-95%。
- 4. 如权利要求1所述的方法,其中所述疫苗的形式选自重组蛋白疫苗、重组基因疫苗、重组病毒疫苗、基因修饰疫苗及稳定转化共生菌。
- 5. 一种制备肿瘤疫苗的方法, 该方法包括:
- 1) 分析动物体内的肿瘤细胞的特异抗原;
- 2) 获得编码特异抗原的多核苷酸序列;
- 3) 获得与该多核苷酸序列有足够差异的多核苷酸序列:
- 4) 利用步骤3) 中所得的多核苷酸序列制备疫苗。
- 6. 如权利要求 5 所述的方法,其中所述肿瘤细胞发生在动物体内。
- 7. 如权利要求 5 所述的方法, 其中所述肿瘤细胞来源于动物体。
 - 8. 如权利要求6或7所述的方法,其中所述动物体包括人体。
 - 9. 如权利要求 5 所述的方法,其中步骤 1) 所述的多核苷酸序列与步骤 3) 所述的多核苷酸序列的差异,以它们所编码的多肽序列而言,同源性为 30%-95%。
 - 10. 如权利要求 5 所述的方法,其中所述疫苗的形式选自重组蛋白疫苗、重组基因疫苗、重组病毒疫苗、基因修饰疫苗及稳定转化共生菌。
 - 11. 一种制备 EGFR 疫苗的方法, 该方法包括:
 - 1) 分析动物体内的肿瘤细胞的 EGFR 抗原:

- 2) 获得编码特异抗原的多核苷酸序列:
- 3) 获得与该多核苷酸序列有足够差异的多核苷酸序列;
- 4) 利用步骤 3) 中所得的多核苷酸序列制备疫苗。
- 12. 如权利要求 11 所述的方法, 其中所述肿瘤细胞发生在动物体内。
- 13. 如权利要求 11 所述的方法, 其中所述肿瘤细胞来源于动物体。
- 14. 如权利要求 11 所述的方法,其中步骤 1) 所述的多核苷酸序列与步骤 3) 所述的多核苷酸序列的差异,以它们所编码的多肽序列而言,同源性为 30% 95%。
- 15. 如权利要求 11 所述的方法,其中所述疫苗的形式选自选自重组蛋白疫苗、重组基因疫苗、重组病毒疫苗、基因修饰疫苗及稳定转化共生菌。
- 17. 一种核酸疫苗,其中含有的多核苷酸序列所编码的多肽与病原体中特定抗原的多核苷酸序列所编码的多肽的同源性为 30-95%。
- 18. 一种表皮生长因子受体(EGFR)核酸疫苗,其中含有的多核苷酸序列编码的表皮生长因子受体与生物体内的该表皮生长因子受体中的氨基酸序列的同源性为 30-95%。
- 19. 一种表皮生长因子受体(EGFR)核酸疫苗,其中含有的多核苷酸序列编码的表皮生长因子受体与人体内的该表皮生长因子受体中的氨基酸序列的同源性为30-95%。
- 20. 一种制备用于治疗人体肿瘤的 EGFR 疫苗的方法,该方法包括:
- 1) 从异种生物获得编码 EGFR 的多核苷酸序列; 和
- 2) 利用步骤 1) 中所得的多核苷酸序列制备疫苗。
- 21. 如权利要求 20 所述的方法,其中所述异种生物是除人之外的其他生物,如小鼠、大鼠、鸡、鲐、果蝇等。
- 22. 如权利要求 20 所述的方法, 其中所述从异种生物获得编码 EGFR 的多核苷酸序列选自 SEQ.ID.No.13, SEQ.ID.No.15. SEQ.ID.No.17, SEQ.ID.No.19, SEQ.ID.No. 21, SEQ.ID. No. 23, SEQ.ID. No.24 和 SEQ.ID.26。

23. 如权利要求 20 所述的方法,其中所述疫苗的形式选自选自重组蛋白疫苗、重组基因疫苗、重组病毒疫苗、基因修饰疫苗及稳定转化共生菌。

- 24. 如权利要求 20 的方法, 其中人体肿瘤选自过量表达 EGFR 分子的肺癌、乳腺癌、卵巢癌、结肠癌、前列腺癌、胃癌、膀胱癌、头颈部鳞癌和胶质瘤等。
- 25. 一种制备用于治疗人体肿瘤的 EGFR 疫苗的方法,该方法包括:
- 1) 修饰人 EGFR 的多核苷酸序列: 和
- 2) 从步骤1) 中所得的多核苷酸序列中选择修饰后的多核苷酸序列制备疫苗。
- 26. 如权利要求 25 的方法, 其中所述人 EGFR 的多核苷酸序列选自 SEQ. ID. No. 1, SEQ. ID. No. 3, SEQ. ID. No. 5, SEQ. ID. No. 7, SEQ. ID. No. 9 和 SEQ. ID. No. 11。
- 27. 如权利要求 25 的方法,其中所述修饰方法选自易错 PCR、改组、寡核苷酸介导的诱变、装配 PCR、有性 PCR 诱变、体内诱变、盒式诱变、递归集团诱变、指数集团诱变、位点特异性诱变、基因再装配、GSSM 及其任意组合。
- 28. 如权利要求 25 所述的方法,其中所述疫苗的形式选自选自重组蛋白疫苗、重组基因疫苗、重组病毒疫苗、基因修饰疫苗及稳定转化共生菌。
- 29. 如权利要求 25 的方法,其中人体肿瘤选自过量表达 EGFR 分子的肺癌、乳腺癌、卵巢癌、结肠癌、前列腺癌、胃癌、膀胱癌、头颈部鳞癌和胶质瘤等。
- 30. 一种表皮生长因子受体多肽疫苗,其中含有的表皮生长因子受体多肽与人体内的该表皮生长因子受体中的氨基酸序列的同源性为30-95%。

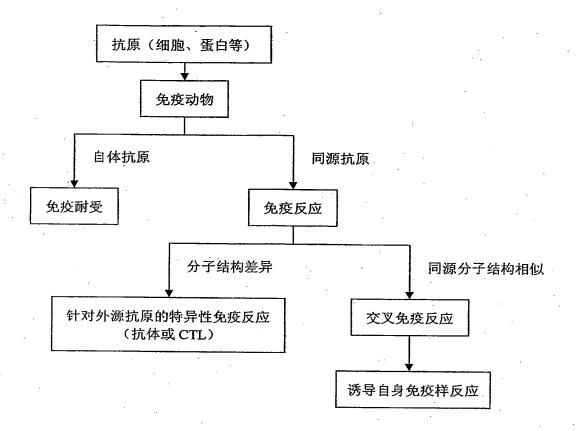
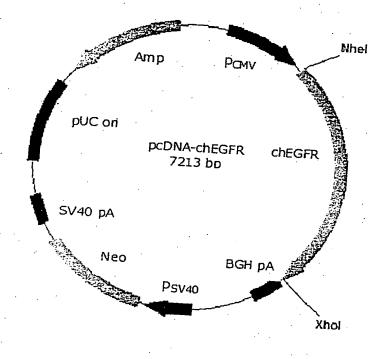
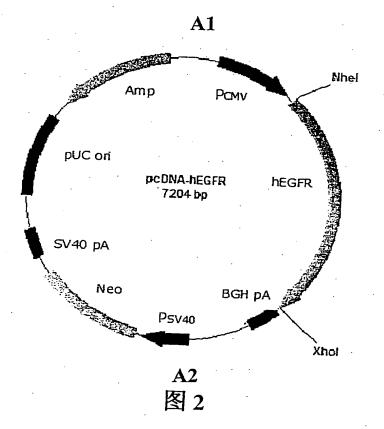
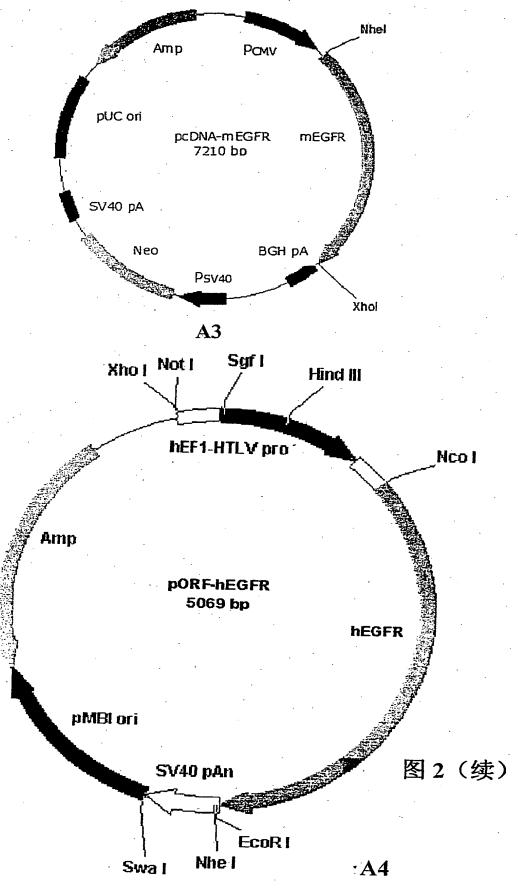
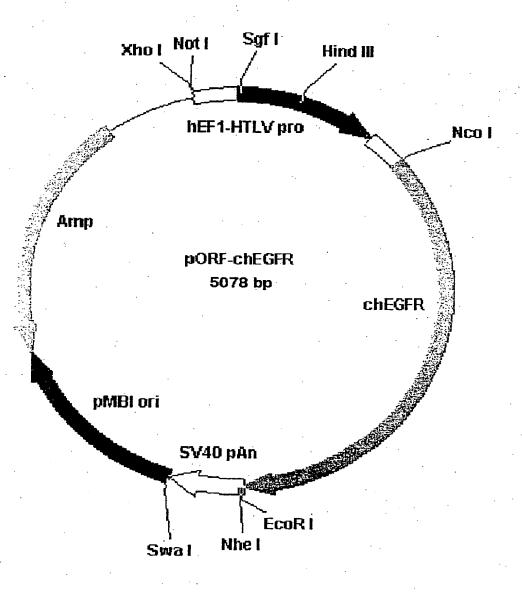


图 1



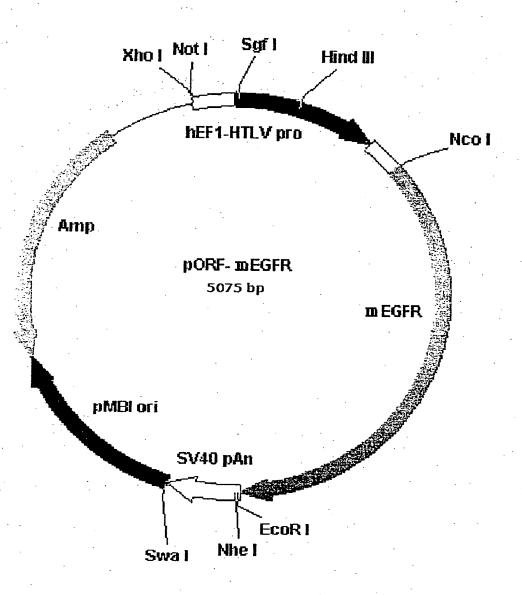






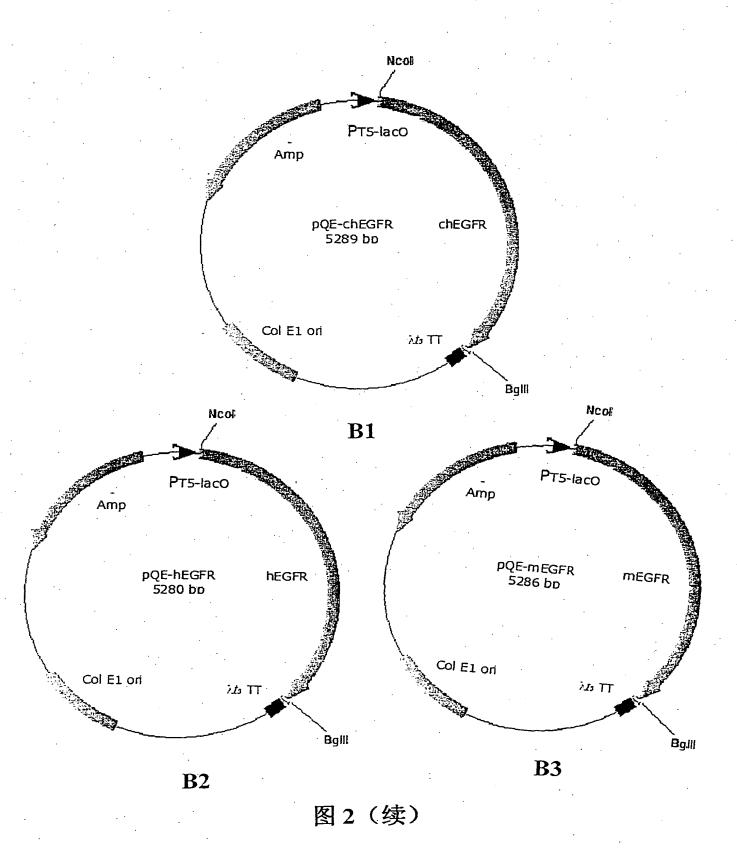
A5

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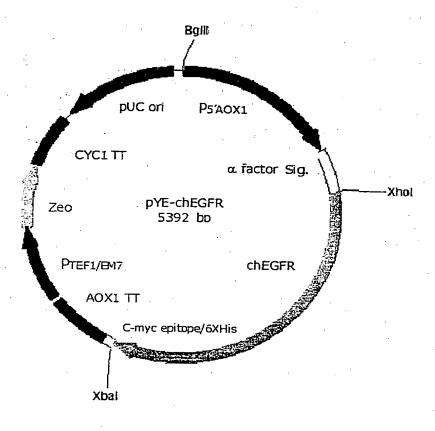


A6

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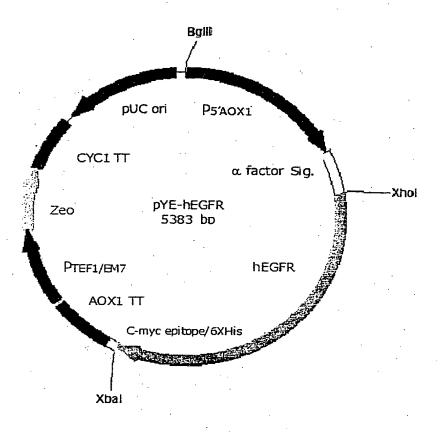


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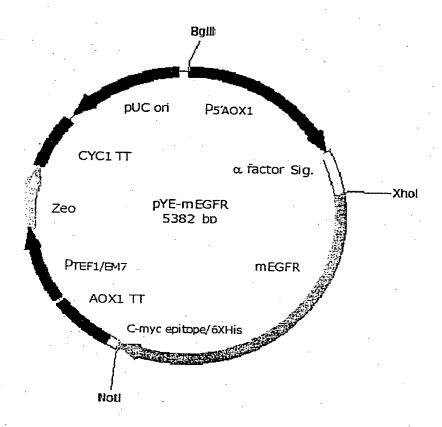
C1

图 2 (续)



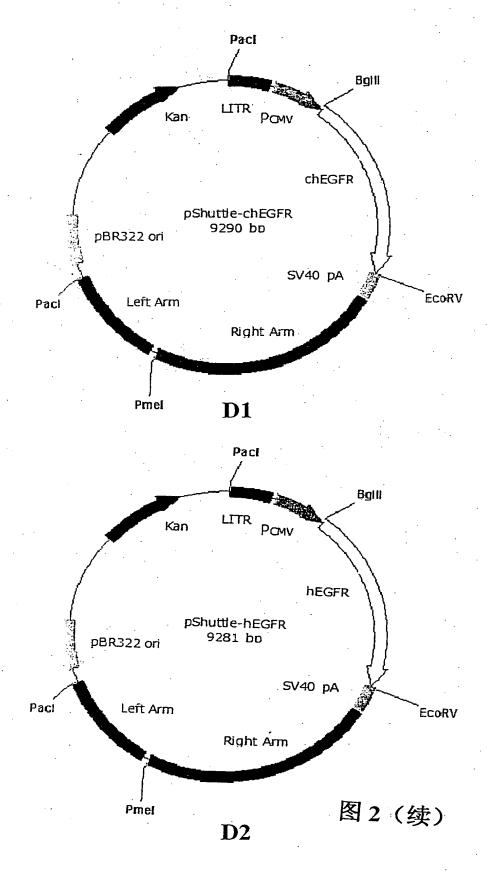
C2

图 2 (续)



C3

图 2 (续)



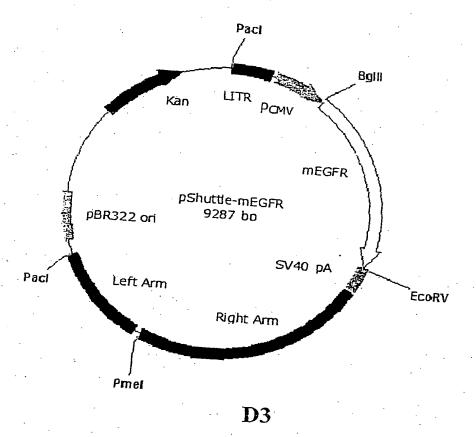
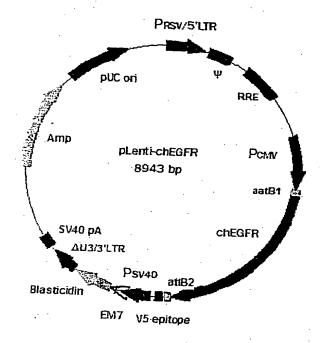
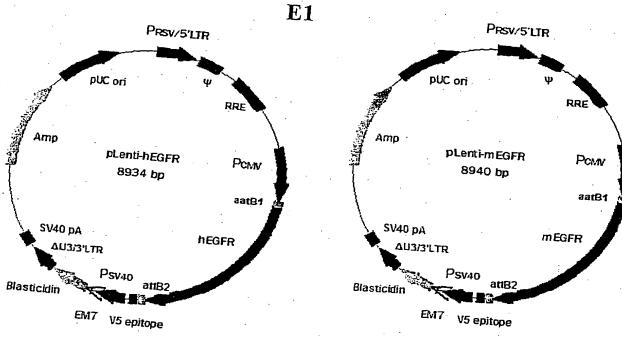


图2(续)



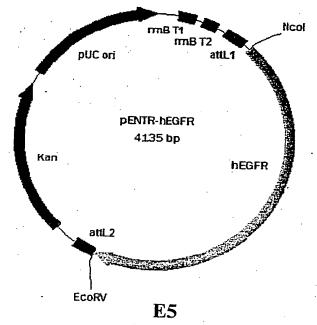


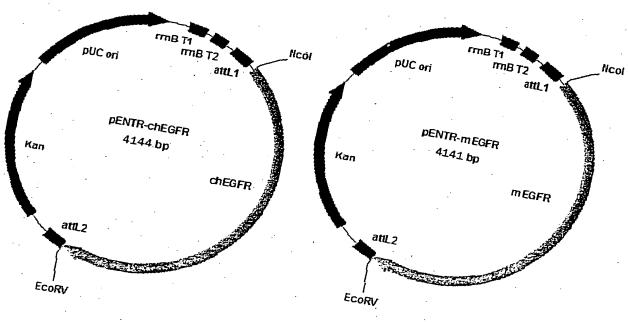
E2

图 2 (续)

E3

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E4 图 2 (续) **E6**

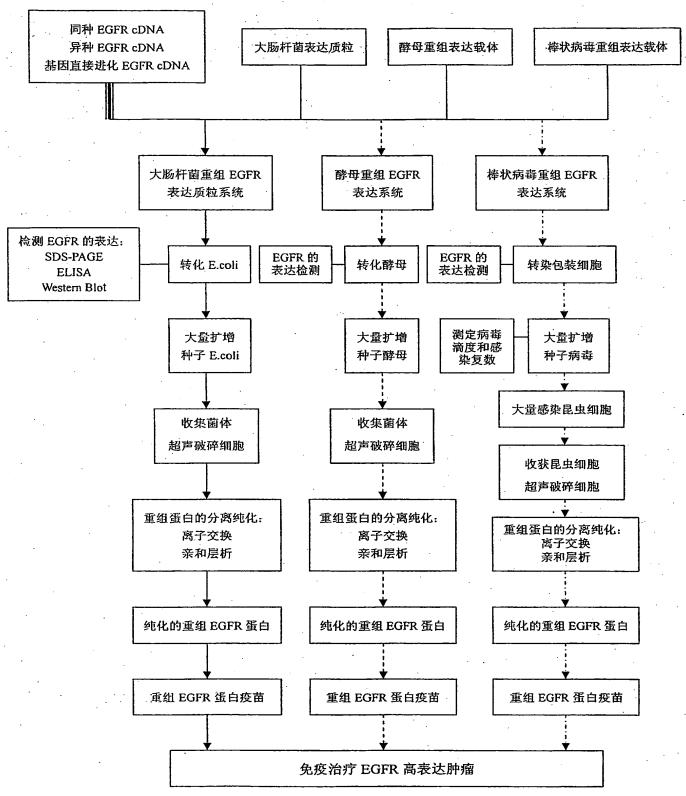


图 3

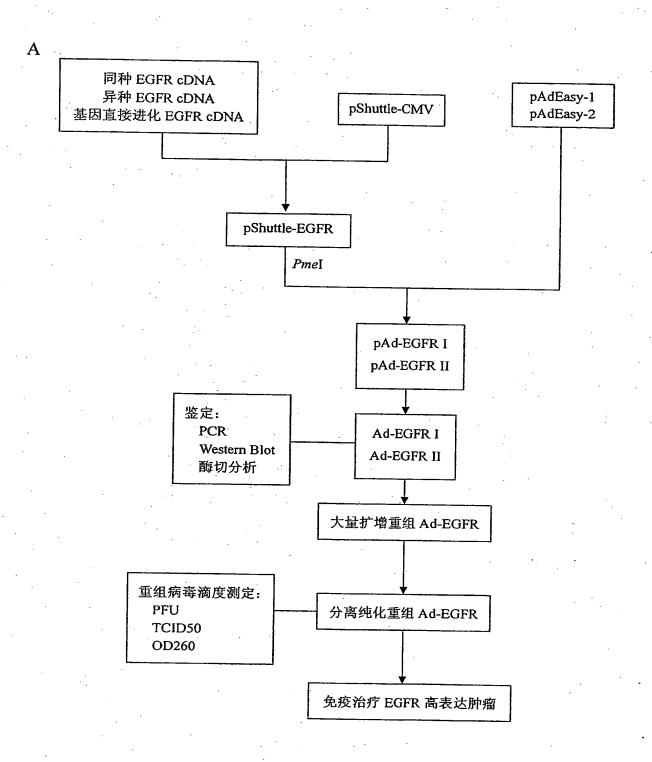
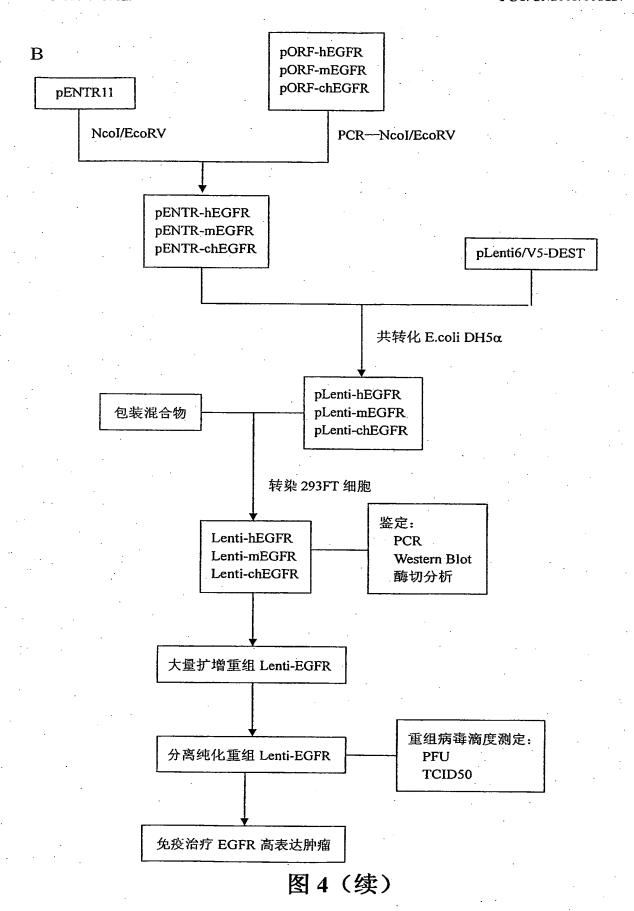
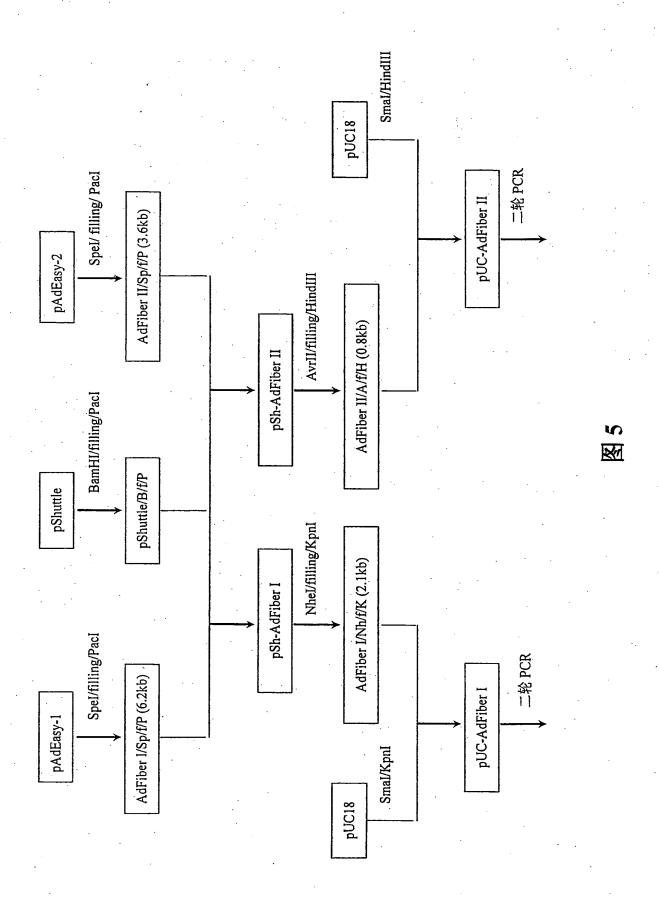
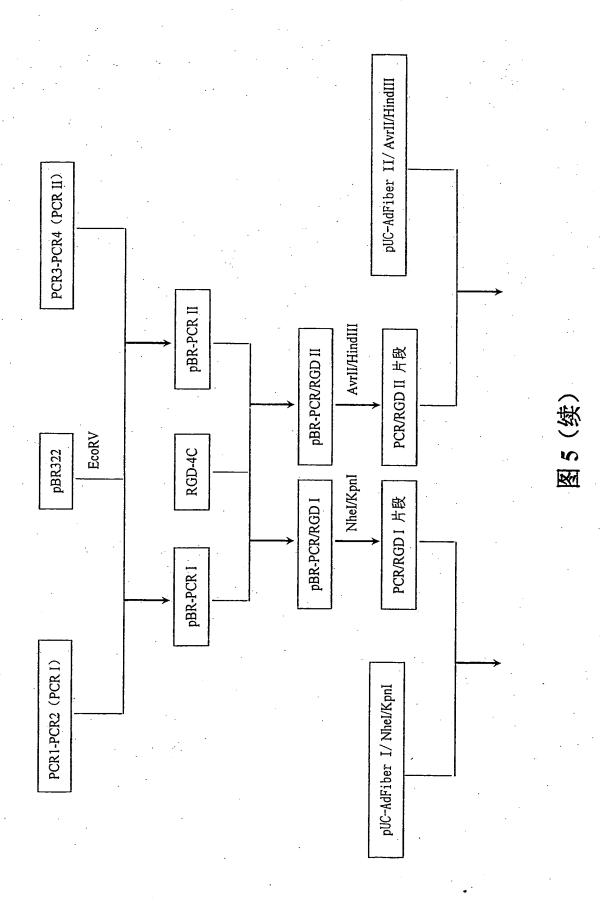


图 4

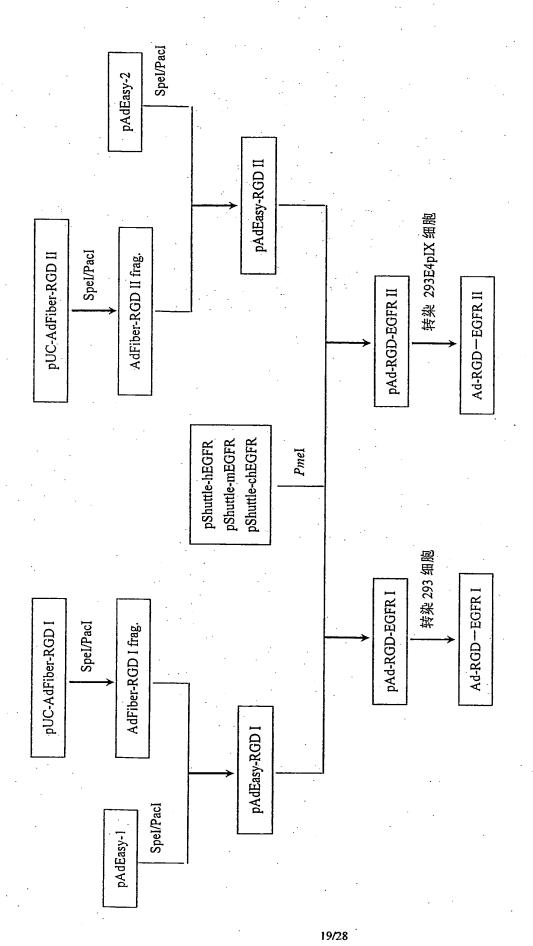


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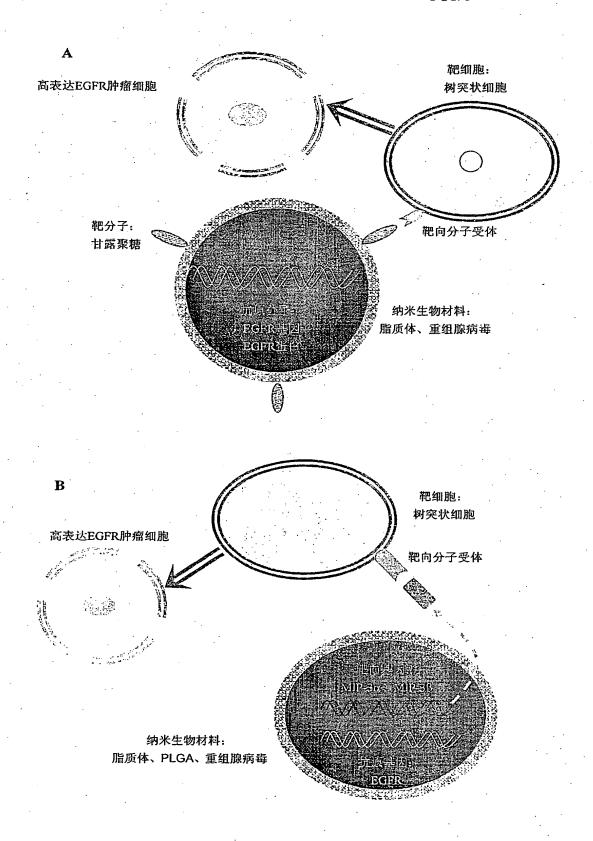


图 6

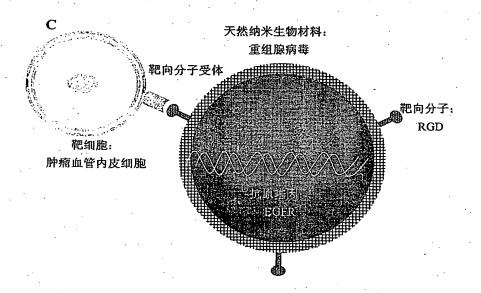


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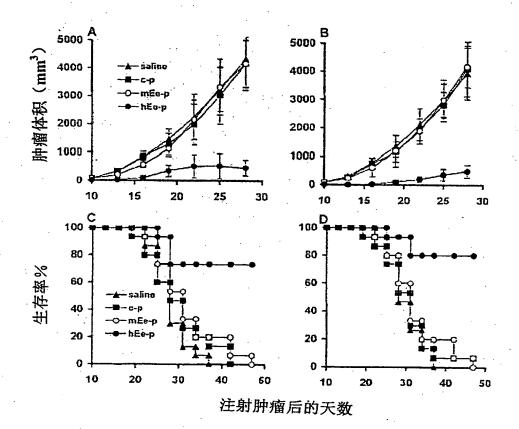


图 7

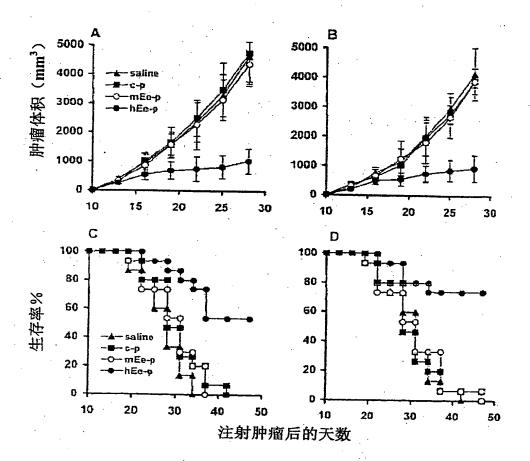
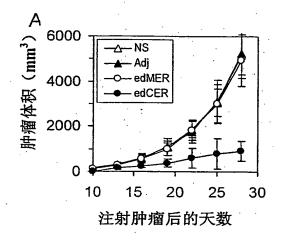
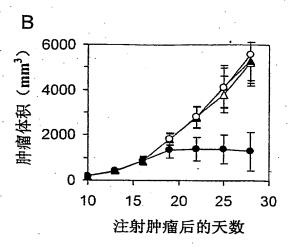


图 8





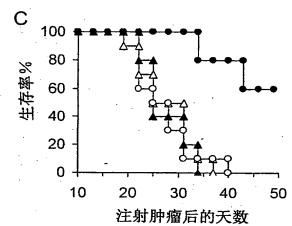
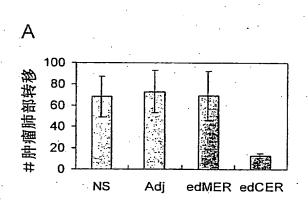


图 9



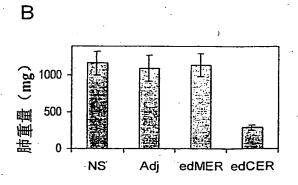


图 10

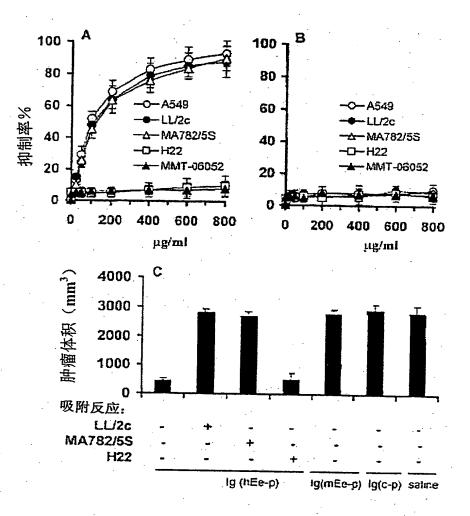
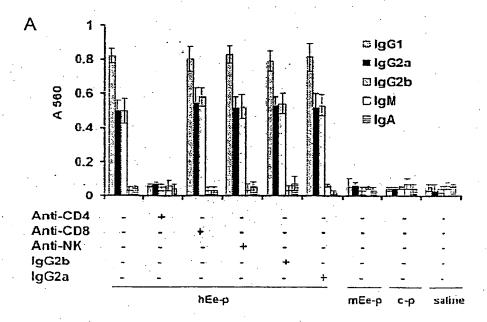


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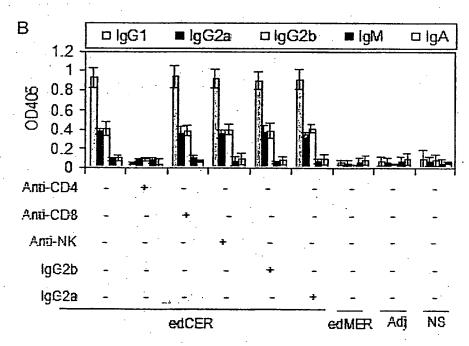


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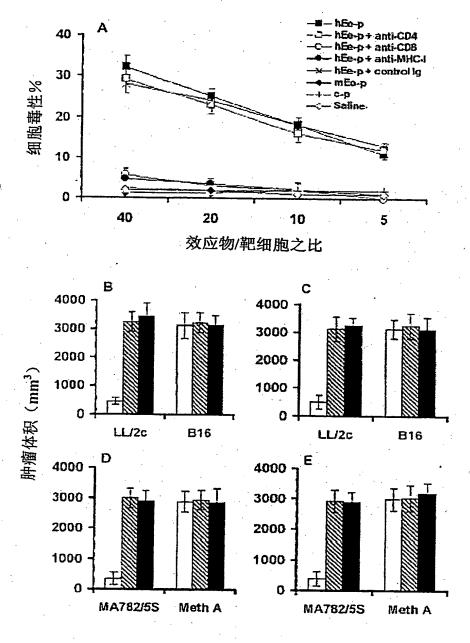


图 13

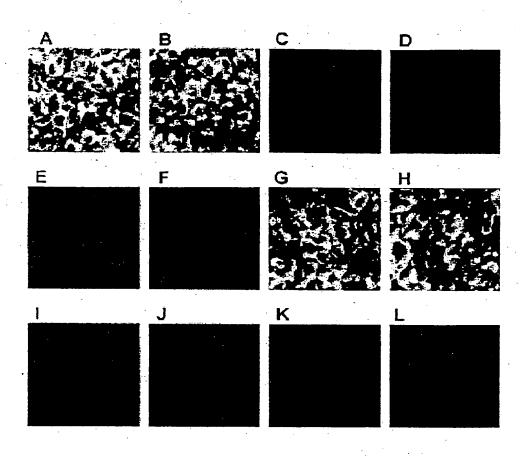


图 14

序列表

<110> 深圳市清华源兴生物医药科技有限公司

<120> 疫苗制备方法和抗肿瘤疫苗

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Gly Thr Ser Asn Lys Leu Thr Gln Leu Gly Thr Phe Glu Asp His Phe
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His Gly Ala Val Arg Phe Ser Asn Asn Pro Ala Leu Cys Asn Val Glu 145 150 155 160

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Ser Cys Pro Asn Gly Ser Cys Trp Gly Ala Gly Glu Glu Asn Cys Gln 195 200 205

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ggc (Gly T	acg Thr 35	Ser	aac Asn	aag Lys	ctc Leu 40	acg Thr	cag Gln	Leu	ggc I Gly 45	act t Thr	tt ga Phe	aa ga Glu .	at car Asp I	t ttt His Ph	144 e	,
ctc a Leu : 50	Ser	ctc (Leu	cag ı Gir	agg Arg 55	Met	tc aa Phe	Asr	ac t n As 60	gt ga n Cy	ag gt /s Gl	tg gt u Va	c ctt I Val	ggg Leu	aat Gly A	192 sn	•
ttg g Leu (65	jaa Glu	att i	Thr	tat g Tyr ' 70	itg ca Val G	ag ag SIn A	gga rgA 75	at t .sn	at ga Tyr <i>l</i>	at cti Asp L	t tcc Leu S 80	ttc t Ser P	ta aa he L	ag eu Lys	240 5	
acc a Thr I	atc (le (3In (gag Glu 35	gtg Val A	gct (Ala G	ggt t ly Ty 90	at g ⁄r Va	tc c	tc at eu Il	tt gc e Ala 95	c ctc a Leu	aac Asn	aca Thr	gtg Val	288	-
gag (Glu /	cga Arg	att Ile I 100	Pro	ttg g Leu	Gļu A	iac ci Asn L 105	tg ca .eu (ag a Gln	Ile I	tc ag le Ar .10	ga gg g Gl	ga aa y Ası	it ato n Me	tac t Tyr	330	6
tac g Tyr (aa Slu 11	Asn	tcc Ser	tat g Tyr	cc tt Ala I 120	Leu A	a gt Ala N	c tta /al	a tct Leu 125	Ser A	tat q Asn T	gat g Tyr A	ca a sp A	at Ia Asr	384 1	
Lys 7	acc Thr 30	gga Gly	ctg Leu	Lys	gag Glu 1 35	ctg Leu I	ccc Pro I	atg Met 14	Arg	aat Asn	tta c Leu	ag g Gln	aa at Glu I	tc ctg le Lei	43 1	32
cat g His G 145	gc Sly /	gcc Ala \	Val /	cgg Arg I 150	he S	gc a Ser A	sn A	ac (\sn 55	cct g Pro	cc ct Ala l	tg tg Leu (16	ys A	gtg Isn V	gag 'al Glu	48 I	0
agc a Ser I	atc (3ln 7	tgg Trp / .65	cgg Arg /	gac Asp I	ata g le Va 17	al Se	igc er S	agt (er A	gac t sp Pl 17	he Le	cage eu Se	c aac er As	: atg n Met	528	3
tcg a Ser N	1et	gac Asp 180	Phe	ag a e Gir	Asn	ac ct His 185	g gg Leu	gc a Gly	∕ Ser	gc ca Cys 90	aa aa GIn	ig tg Lys	t gat Cys /	cca Asp Pi	576 ro	5
agc t Ser C	gt d Cys 19	Pro	at g Asn	ggg a Gly	agc t Ser 200	Cys :	ggg Trp	gt g Gly	gca g Ala 205	ga g Gly (gag g Glu (jag a Slu A	ac to sn C	gc cag ys Gli) 6 1	24
aaa c Lys L 21	eu '	acc Thr	aaa Lys	Ile I	atc to le Cy L5	gt gc /s Ala	cc ca a Gl	ig c n G 22	In C	gc to ys Se	c gg er Gl	g cg y Arq	tgc Cys	cgt Arg	672	2
ggc a Gly L	ag ys S	tcc Ser	ccc Pro	agt (Ser	gac t Asp (gc to Cys (gc ca Cys	ac a His	ac c Asn	ag t <u>o</u> Gln	gt gc Cyș	t gca Ala A 12	la G	tgc ly Cys	720 i)

	• • •		•	
225	230	235	240	
aca ggc ccc c Thr Gly Pro A 24	rg Glu Ser Asp C	ys Leu Val Cys	gc aaa ttc cga gac Arg Lys Phe Arg As 5	768 p
gaa gcc acg t Glu Ala Thr C 260	gc aag gac acc t ys Lys Asp Thr C 265	gc ccc cca ctc a cys Pro Pro Leu I 270	tg ctc tac aac ccc Met Leu Tyr Asn Pr	.816 o
acc acg tac ca Thr Thr Tyr G 275	ag atg gat gtg aa In Met Asp Val A 280	ac ccc gag ggc a sn Pro Glu Gly I 285	aaa tac agc ttt ggt _ys Tyr Ser Phe Gly	864
gcc acc tgc gt Ala Thr Cys V 290	tg aag aag tgt co al Lys Lys Cys Pi 295	cc cgt aat tat gtg ro Arg Asn Tyr \ 300	g gtg aca gat cac /al Val Thr Asp His	912
ggc tcg tgc gt Gly Ser Cys V 305	cc cga gcc tgt gg 'al Arg Ala Cys G 310	g gcc gac agc ta lly Ala Asp Ser T 315	at gag atg gag gaa Tyr Glu Met Glu Glu 320	960
gac ggc gtc c Asp Gly Val A 32	rg Lys Cys Lys L	ys Cys Glu Gly I	cct tgc cgc aaa gtg Pro Cys Arg Lys Val 5	1008
tgt aac gga a Cys Asn Gly I 340	ta ggt att ggt ga le Gly Ile Gly Glu 345	a ttt aaa gac tc I Phe Lys Asp So 350	a ctc tcc ata aat er Leu Ser Ile Asn	1056
gct acg aat at Ala Thr Asn II 355	t aaa cac ttc aaa e Lys His Phe Ly 360	a aac tgc acc tcc s Asn Cys Thr S 365	atc agt ggc gat er Ile Ser Gly Asp	1104
ctc cac atc ctc Leu His Ile Le 370	g ccg gtg gca ttt u Pro Val Ala Pho 375	agg ggt gac tcc e Arg Gly Asp S 380	ttc aca cat act er Phe Thr His Thr	1152
cct cct ctg gal Pro Pro Leu A 385	t cca cag gaa ctg sp Pro Gin Giu L 390	gat att ctg aaa eu Asp Ile Leu L 395	acc gta aag gaa ys Thr Val Lys Glu 400	1200
atc aca ggg tt Ile Thr Gly Ph 40	e Leu Leu Ile Gli	n Ala Trp Pro Gli	aac agg acg gac u Asn Arg Thr Asp 5	1248
ctc cat gcc ttt Leu His Ala Ph 420	gag aac cta gaa ne Glu Asn Leu G 425	atc ata cgc ggo Blu Ile Ile Arg Gl 430	agg acc aag caa y Arg Thr Lys Gln	1296
cat ggt cag tti His Gly Gln Ph 435	t tct ctt gca gtc q ne Ser Leu Ala Vi 440	gtc agc ctg aac a al Val Ser Leu A 445	ata aca tcc ttg 1 sn Ile Thr Ser Leu	344
gga tta cgc tc Gly Leu Arg S 450	c ctc aag gag at er Leu Lys Glu II 455	a agt gat gga ga e Ser Asp Gly A 460	at gtg ata att tca sp Val Ile Ile Ser	1392
gga aac aaa a Gly Asn Lys As 465	at ttg tgc tat gc sn Leu Cys Tyr A 470	a aat aca ata aa lla Asn Thr Ile A 475	c tgg aaa aaa ctg sn Trp Lys Lys Leu 480	1440

ttt ggg acc tcc ggt cag aaa acc aaa att ata agc aac aga ggt gaa

1488

Phe Gly Thr Ser Gly Gln Lys Thr Lys Ile Ile Ser Asn Arg Gly Glu 490 aac agc tgc aag gcc aca ggc cag gtc tgc cat gcc ttg tgc tcc ccc 1536 Asn Ser Cys Lys Ala Thr Gly Gln Val Cys His Ala Leu Cys Ser Pro 510 gag ggc tgc tgg ggc ccg gag ccc agg gac tgc gtc tct tgc cgg aat 1584 Glu Gly Cys Trp Gly Pro Glu Pro Arg Asp Cys Val Ser Cys Arg Asn gtc agc cga ggc agg gaa tgc gtg gac aag tgc aac ctt ctg gag ggt 1632 Val Ser Arg Gly Arg Glu Cys Val Asp Lys Cys Asn Leu Leu Gly Gly 535 540 gag cca agg gag ttt gtg gag aac tct gag tgc ata cag tgc cac cca 1680 Glu Pro Arg Glu Phe Val Glu Asn Ser Glu Cys Ile Gln Cys His Pro 550 gag tgc ctg cct cag gcc atg aac atc acc tgc aca gga cgg gga cca 1728 Glu Cys Leu Pro Gln Ala Met Asn Ile Thr Cys Thr Gly Arg Gly Pro 570 gac aac tgt atc cag tgt gcc cac tac att gac ggc ccc cac tgc gtc Asp Asn Cys Ile Gln Cys Ala His Tyr Ile Asp Gly Pro His Cys Val 585 590 aag acc tgc ccg gca gga gtc atg gga gaa aac aac acc ctg gtc tgg 1824 Lys Thr Cys Pro Ala Gly Val Met Gly Glu Asn Asn Thr Leu Val Trp 600 605 aag tac gca gac gcc ggc cat gtg tgc cac ctg tgc cat cca aac tgc Lys Tyr Ala Asp Ala Gly His Val Cys His Leu Cys His Pro Asn Cys 615 620 acc tac gga tgc act ggg cca ggt ctt gaa ggc tgt cca acg aat gga 1920 Thr Tyr Gly Cys Thr Gly Pro Gly Leu Glu Gly Cys Pro Thr Asn Gly age tac ata gtg tet cae ttt eea aga tea tte tac aag atg tea gtg 1968 Ser Tyr Ile Val Ser His Phe Pro Arg Ser Phe Tyr Lys Met Ser Val 650 cac tga 1974 His <210> 4 <211> 657 <212> PRT <213> 人(homo sapiens)

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- Gly Thr Ser Asn Lys Leu Thr Gln Leu Gly Thr Phe Glu Asp His Phe 35 40 45
- Leu Ser Leu Gln Arg Met Phe Asn Asn Cys Glu Val Val Leu Gly Asn 50 55 60
- Leu Glu Ile Thr Tyr Val Gln Arg Asn Tyr Asp Leu Ser Phe Leu Lys 65 70 75 80
- Thr Ile Gin Giu Val Ala Gly Tyr Val Leu Ile Ala Leu Asn Thr Val 85 90 95
- Glu Arg Ile Pro Leu Glu Asn Leu Gln Ile Ile Arg Gly Asn Met Tyr 100 105 110
- Tyr Glu Asn Ser Tyr Ala Leu Ala Val Leu Ser Asn Tyr Asp Ala Asn 115 120 125
- Lys Thr Gly Leu Lys Glu Leu Pro Met Arg Asn Leu Gln Glu Ile Leu 130 135 140
- His Gly Ala Val Arg Phe Ser Asn Asn Pro Ala Leu Cys Asn Val Glu 145 150 155 160
- Ser Ile Gin Trp Arg Asp Ile Val Ser Ser Asp Phe Leu Ser Asn Met 165 170 175
- Ser Met Asp Phe Gln Asn His Leu Gly Ser Cys Gln Lys Cys Asp Pro 180 185 190
- Ser Cys Pro Asn Gly Ser Cys Trp Gly Ala Gly Glu Glu Asn Cys Gln 195 200 205
- Lys Leu Thr Lys Ile Ile Cys Ala Gln Gln Cys Ser Gly Arg Cys Arg 210 215 220
- Gly Lys Ser Pro Ser Asp Cys Cys His Asn Gln Cys Ala Ala Gly Cys 225 230 235 240
- Thr Gly Pro Arg Glu Ser Asp Cys Leu Val Cys Arg Lys Phe Arg Asp 245 250 255
- Glu Ala Thr Cys Lys Asp Thr Cys Pro Pro Leu Met Leu Tyr Asn Pro 260 265 270

- Thr Thr Tyr Gln Met Asp Val Asn Pro Glu Gly Lys Tyr Ser Phe Gly 275 280 285
- Ala Thr Cys Val Lys Lys Cys Pro Arg Asn Tyr Val Val Thr Asp His 290 295 300
- Gly Ser Cys Val Arg Ala Cys Gly Ala Asp Ser Tyr Glu Met Glu Glu 305 310 315 320
- Asp Gly Val Arg Lys Cys Lys Cys Glu Gly Pro Cys Arg Lys Val 325 330 335
- Cys Asn Gly Ile Gly Ile Gly Glu Phe Lys Asp Ser Leu Ser Ile Asn 340 345 350
- Ala Thr Asn Ile Lys His Phe Lys Asn Cys Thr Ser Ile Ser Gly Asp 355 360 365
- Leu His Ile Leu Pro Val Ala Phe Arg Gly Asp Ser Phe Thr His Thr 370 375 380
- Pro Pro Leu Asp Pro Gln Glu Leu Asp Ile Leu Lys Thr Val Lys Glu 385 390 395 400
- Ile Thr Gly Phe Leu Leu Ile Gln Ala Trp Pro Glu Asn Arg Thr Asp 405 410 415
- Leu His Ala Phe Glu Asn Leu Glu Ile Ile Arg Gly Arg Thr Lys Gln 420 425 430
- His Gly Gln Phe Ser Leu Ala Val Val Ser Leu Asn Ile Thr Ser Leu 435 440 445
- Gly Leu Arg Ser Leu Lys Glu Ile Ser Asp Gly Asp Val Ile Ile Ser 450 455 460
- Gly Asn Lys Asn Leu Cys Tyr Ala Asn Thr Ile Asn Trp Lys Lys Leu 465 470 475 480
- Phe Gly Thr Ser Gly Gln Lys Thr Lys Ile Ile Ser Asn Arg Gly Glu 485 490 495
- Asn Ser Cys Lys Ala Thr Gly Gln Val Cys His Ala Leu Cys Ser Pro 500 505 510
- Glu Gly Cys Trp Gly Pro Glu Pro Arg Asp Cys Val Ser Cys Arg Asn

520

525

Val Ser Arg Gly Arg Glu Cys Val Asp Lys Cys Asn Leu Leu Glu Gly 530 535 540

Glu Pro Arg Glu Phe Val Glu Asn Ser Glu Cys Ile Gln Cys His Pro 545 550 555 560

Glu Cys Leu Pro Gln Ala Met Asn IIe Thr Cys Thr Gly Arg Gly Pro 565 570 575

Asp Asn Cys Ile Gln Cys Ala His Tyr Ile Asp Gly Pro His Cys Val 580 585 590

Lys Thr Cys Pro Ala Gly Val Met Gly Glu Asn Asn Thr Leu Val Trp 595 600 605

Lys Tyr Ala Asp Ala Gly His Val Cys His Leu Cys His Pro Asn Cys 610 615 620

Thr Tyr Gly Cys Thr Gly Pro Gly Leu Glu Gly Cys Pro Thr Asn Gly 625 630 635 640

Ser Tyr Ile Val Ser His Phe Pro Arg Ser Phe Tyr Lys Met Ser Val 645 650 655

His

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ggc acg agt aac aag ctc acg cag ttg ggc act ttt gaa gat cat ttt Gly Thr Ser Asn Lys Leu Thr Gln Leu Gly Thr Phe Glu Asp His Phe 35 40 45	144 e
ctc agc ctc cag agg atg ttc aat aac tgt gag gtg gtc ctt ggg aat Leu Ser Leu Gln Arg Met Phe Asn Asn Cys Glu Val Val Leu Gly As 50 55 60	192 in
ttg gaa att acc tat gtg cag agg aat tat gat ctt tcc ttc tta aag Leu Glu Ile Thr Tyr Val Gln Arg Asn Tyr Asp Leu Ser Phe Leu Lys 65 70 75 80	240
acc atc cag gag gtg gct ggt tat gtc ctc att gcc ctc aac aca gtg Thr Ile Gln Glu Val Ala Gly Tyr Val Leu Ile Ala Leu Asn Thr Val 85 90 95	288
gag cga att cct ttg gaa aac ctg cag atc atc aga gga aat atg tac Glu Arg Ile Pro Leu Glu Asn Leu Gln Ile Ile Arg Gly Asn Met Tyr 100 105 110	-336
tac gaa aat tcc tat gcc tta gca gtc tta tct aac tat gat gca aat Tyr Glu Asn Ser Tyr Ala Leu Ala Val Leu Ser Asn Tyr Asp Ala Asn 115 120 125	384
aaa acc gga ctg aag gag ctg ccc atg aga aat tta cag gaa atc ctg Lys Thr Gly Leu Lys Glu Leu Pro Met Arg Asn Leu Gln Glu Ile Leu 130 135 140	432
cat ggc gcc gtg cgg ttc agc aac aac cct gcc ctg tgc aac gtg gag His Gly Ala Val Arg Phe Ser Asn Asn Pro Ala Leu Cys Asn Val Glu 145 150 155 160	480
agc atc cag tgg cgg gac ata gtc agc agt gac ttt ctc agc aac atg Ser Ile Gin Trp Arg Asp Ile Val Ser Ser Asp Phe Leu Ser Asn Met 165 170 175	528
tcg atg gac ttc cag aac cac ctg ggc agc tgc caa aag tgt gat cca Ser Met Asp Phe Gln Asn His Leu Gly Ser Cys Gln Lys Cys Asp Pro 180 185 190	576)
agc tgt ccc aat ggg agc tgc tgg ggt gca gga gag gag aac tgc cag Ser Cys Pro Asn Gly Ser Cys Trp Gly Ala Gly Glu Glu Asn Cys Gln 195 200 205	624
aaa ctg acc aaa atc atc tgt gcc cag cag tgc tcc ggg cgc tgc cgt Lys Leu Thr Lys Ile Ile Cys Ala Gln Gln Cys Ser Gly Arg Cys Arg 210 215 220	672
ggc aag tcc ccc agt gac tgc tgc cac aac cag tgt gct gca ggc tgc Gly Lys Ser Pro Ser Asp Cys Cys His Asn Gln Cys Ala Ala Gly Cys 225 230 235 240	720
aca ggc ccc cgg gag agc gac tgc ctg gtc tgc cgc aaa ttc cga gac Fhr Gly Pro Arg Glu Ser Asp Cys Leu Val Cys Arg Lys Phe Arg Asp 245 250 255	768
gaa gcc acg tgc aag gac acc tgc ccc cca ctc atg ctc tac aac ccc Glu Ala Thr Cys Lys Asp Thr Cys Pro Pro Leu Met Leu Tyr Asp Pro	816

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4	Oυ

acc acg tac cag atg gat gtg aac ccc gag ggc aaa tac agc ttt gg Thr Thr Tyr Gln Met Asp Val Asn Pro Glu Gly Lys Tyr Ser Phe G 275 280 285	t 864 Gly
gcc acc tgc gtg aag aag tgt ccc cgt aat tat gtg gtg aca gat cac Ala Thr Cys Val Lys Lys Cys Pro Arg Asn Tyr Val Val Thr Asp H 290 295 300	912 is
ggc tcg tgc gtc cga gcc tgt ggg gcc gac agc tat gag atg gag ga Gly Ser Cys Val Arg Ala Cys Gly Ala Asp Ser Tyr Glu Met Glu G 305 310 315 320	ia 960 lu
gac ggc gtc cgc aag tgt aag aag tgc gaa ggg cct tgc cgc aaa g Asp Gly Val Arg Lys Cys Lys Lys Cys Glu Gly Pro Cys Arg Lys V 325 330 335	tg 1008 'al
tgt aac gga ata ggt att ggt gaa ttt aaa gac tca ctc tcc ata aat Cys Asn Gly Ile Gly Ile Gly Glu Phe Lys Asp Ser Leu Ser Ile Asi 340 345 350	1056 1
gct acg aat att aaa cac ttc aaa aac tgc acc tcc atc agt ggc gat Ala Thr Asn Ile Lys His Phe Lys Asn Cys Thr Ser Ile Ser Gly Asp 355 360 365	1104
ctc cac atc ctg ccg gtg gca ttt agg ggt gac tcc ttc aca cat act Leu His Ile Leu Pro Val Ala Phe Arg Gly Asp Ser Phe Thr His Th 370 375 380	1152 r
cct cct ctg gat cca cag gaa ctg gat att ctg aaa acc gta aag gaa Pro Pro Leu Asp Pro Gln Glu Leu Asp Ile Leu Lys Thr Val Lys Gl 385 390 395 400	1200 u
atc aca ggg ttt ttg ctg att cag gct tgg cct gaa aac agg acg gac Ile Thr Gly Phe Leu Leu Ile Gln Ala Trp Pro Glu Asn Arg Thr Asp 405 410 415	1248
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cat ggt cag ttt tct ctt gca gtc gtc agc ctg aac ata aca tcc ttg His Gly Gln Phe Ser Leu Ala Val Val Ser Leu Asn Ile Thr Ser Leu 435 440 445	1344 I
gga tta cgc tcc ctc aag gag ata agt gat gga gat gtg ata att tca Gly Leu Arg Ser Leu Lys Glu Ile Ser Asp Gly Asp Val Ile Ile Ser 450 455 460	1392
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Glu Gly 51			ro Glu 520	Pro Ar	g Asp 525	•	'al Ser (Cys Arg	Asn	
gtc agc Val Ser 530			lu Cys	Val As						1632
gag cca Glu Pro 545					er Glu					1680
gag tgc Glu Cys		o Gln A	Ala Met				hr Gly A			1728
gac aac Asp Asr					r Ile A					1776
	Cys Pro	Ala C				Asn A		ctg gtc t _eu Val		1824
			Sly His \	Val Cy:				a aac tg o Asn C		1872
					u Lys			c tgc cti Phe Cys		1920
		r Ser	Cys Ası				Gly Ser	tcc cac Val Sei		968
					ı Ser			gg atc c rp Ile P		2016
Ser Leu						/ Trp (agc cac Cys Ser		2064
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cac tga His 705			•			-	211	8	٠	
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Leu Glu Ile Thr Tyr Val Gln Arg Asn Tyr Asp Leu Ser Phe Leu Lys
65 70 75 80

Thr Ile Gin Glu Val Ala Gly Tyr Val Leu Ile Ala Leu Asn Thr Val 85 90 95

Glu Arg Ile Pro Leu Glu Asn Leu Gln Ile Ile Arg Gly Asn Met Tyr 100 105 110

Tyr Glu Asn Ser Tyr Ala Leu Ala Val Leu Ser Asn Tyr Asp Ala Asn 115 120 125

Lys Thr Gly Leu Lys Glu Leu Pro Met Arg Asn Leu Gln Glu Ile Leu 130 135 140

His Gly Ala Val Arg Phe Ser Asn Asn Pro Ala Leu Cys Asn Val Glu 145 150 155 160

Ser Ile Gin Trp Arg Asp Ile Val Ser Ser Asp Phe Leu Ser Asn Met 165 170 175

Ser Met Asp Phe Gin Asn His Leu Gly Ser Cys Gin Lys Cys Asp Pro 180 185 190

Ser Cys Pro Asn Gly Ser Cys Trp Gly Ala Gly Glu Glu Asn Cys Gln 195 200 205

Lys Leu Thr Lys Ile Ile Cys Ala Gln Gln Cys Ser Gly Arg Cys Arg 210 215 220

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- Glu Ala Thr Cys Lys Asp Thr Cys Pro Pro Leu Met Leu Tyr Asn Pro 260 265 270
- Thr Thr Tyr Gln Met Asp Val Asn Pro Glu Gly Lys Tyr Ser Phe Gly 275 280 285
- Ala Thr Cys Val Lys Lys Cys Pro Arg Asn Tyr Val Val Thr Asp His 290 295 300
- Gly Ser Cys Val Arg Ala Cys Gly Ala Asp Ser Tyr Glu Met Glu Glu 305 310 315 320
- Asp Gly Val Arg Lys Cys Lys Lys Cys Glu Gly Pro Cys Arg Lys Val 325 330 335
- Cys Asn Gly Ile Gly Ile Gly Glu Phe Lys Asp Ser Leu Ser Ile Asn 340 345 350
- Ala Thr Asn Ile Lys His Phe Lys Asn Cys Thr Ser Ile Ser Gly Asp 355 360 365
- Leu His Ile Leu Pro Val Ala Phe Arg Gly Asp Ser Phe Thr His Thr 370 375 380
- Pro Pro Leu Asp Pro Gln Glu Leu Asp Ile Leu Lys Thr Val Lys Glu 385 390 395 400
- Ile Thr Gly Phe Leu Leu Ile Gln Ala Trp Pro Glu Asn Arg Thr Asp 405 410 415
- Leu His Ala Phe Glu Asn Leu Glu Ile Ile Arg Gly Arg Thr Lys Gln 420 425 430
- His Gly Gln Phe Ser Leu Ala Val Val Ser Leu Asn Ile Thr Ser Leu 435 440 445
- Gly Leu Arg Ser Leu Lys Glu IIe Ser Asp Gly Asp Val IIe IIe Ser 450 455 460
- Gly Asn Lys Asn Leu Cys Tyr Ala Asn Thr Ile Asn Trp Lys Lys Leu 465 470 475 480
- Phe Gly Thr Ser Gly Gln Lys Thr Lys Ile Ile Ser Asn Arg Gly Glu 485 490 495
- Asn Ser Cys Lys Ala Thr Gly Gln Val Cys His Ala Leu Cys Ser Pro

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Glu Pro Arg Glu Phe Val Glu Asn Ser Glu Cys Ile Gln Cys His Pro 545 550 555 560

Glu Cys Leu Pro Gln Ala Met Asn Ile Thr Cys Thr Gly Arg Gly Pro 565 570 575

Asp Asn Cys Ile Gln Cys Ala His Tyr Ile Asp Gly Pro His Cys Val 580 585 590

Lys Thr Cys Pro Ala Gly Val Met Gly Glu Asn Asn Thr Leu Val Trp 595 600 605

Lys Tyr Ala Asp Ala Gly His Val Cys His Leu Cys His Pro Asn Cys 610 615 620

Thr Tyr Gly Pro Gly Asn Glu Ser Leu Lys Ala Met Leu Phe Cys Leu 625 630 635 640

Phe Lys Leu Ser Ser Cys Asn Gln Ser Asn Asp Gly Ser Val Ser His 645 650 655

Gln Ser Gly Ser Pro Ala Ala Gln Glu Ser Cys Leu Gly Trp Ile Pro 660 665 670

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His 705

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ggc acg agt aac aag ctc acg cag ttg ggc act ttt gaa gat cat ttt Gly Thr Ser Asn Lys Leu Thr Gln Leu Gly Thr Phe Glu Asp His Pt 35 40 45	144 he
ctc agc ctc cag agg atg ttc aat aac tgt gag gtg gtc ctt ggg aat Leu Ser Leu Gln Arg Met Phe Asn Asn Cys Glu Val Val Leu Gly A 50 55 60	192 Asn
ttg gaa att acc tat gtg cag agg aat tat gat ctt tcc ttc tta aag Leu Glu Ile Thr Tyr Val Gln Arg Asn Tyr Asp Leu Ser Phe Leu Ly 65 70 75 80	240 ⁄s
acc atc cag gag gtg gct ggt tat gtc ctc att gcc ctc aac aca gtg Thr Ile Gin Glu Val Ala Gly Tyr Val Leu Ile Ala Leu Asn Thr Val 85 90 95	288
gag cga att cct ttg gaa aac ctg cag atc atc aga gga aat atg tac Glu Arg Ile Pro Leu Glu Asn Leu Gln Ile Ile Arg Gly Asn Met Tyr 100 105 110	336
tac gaa aat tcc tat gcc tta gca gtc tta tct aac tat gat gca aat Tyr Glu Asn Ser Tyr Ala Leu Ala Val Leu Ser Asn Tyr Asp Ala As 115 120 125	384 sn
aaa acc gga ctg aag gag ctg ccc atg aga aat tta cag gaa atc ctc Lys Thr Gly Leu Lys Glu Leu Pro Met Arg Asn Leu Gln Glu Ile Le 130 135 140	g 432 eu
cat ggc gcc gtg cgg ttc agc aac aac cct gcc ctg tgc aac gtg gag His Gly Ala Val Arg Phe Ser Asn Asn Pro Ala Leu Cys Asn Val G 145 150 155 160	480 lu
agc atc cag tgg cgg gac ata gtc agc agt gac ttt ctc agc aac atg Ser Ile Gln Trp Arg Asp Ile Val Ser Ser Asp Phe Leu Ser Asn Me 165 170 175	528 et
tcg atg gac ttc cag aac cac ctg ggc agc tgc caa aag tgt gat cca Ser Met Asp Phe Gln Asn His Leu Gly Ser Cys Gln Lys Cys Asp 180 185 190	576 Pro
agc tgt ccc aat ggg agc tgc tgg ggt gca gga gag gag aac tgc ca Ser Cys Pro Asn Gly Ser Cys Trp Gly Ala Gly Glu Glu Asn Cys G	

	•	,			-
195	200	20	05		
aaa ctg acc aa Lys Leu Thr Ly 210	a atc atc tgt s Ile Ile Cys 215	gcc cag cag Ala Gin Gin 220	tgc tcc ggg Cys Ser Gly	cgc tgc cgt Arg Cys Arg	672
ggc aag tcc ccc Gly Lys Ser Pro 225	c agt gac tgc o Ser Asp Cy 230	tgc cac aad s Cys His As 235	cag tgt gct on Gln Cys A 240	la Ala Gly Cys	720
aca ggc ccc cg Thr Gly Pro Arg 245	g Glu Ser Ası	c tgc ctg gt Cys Leu V 250	c tgc cgc aa al Cys Arg L 255	a ttc cga gac ys Phe Arg Asp	768
gaa gcc acg tg Glu Ala Thr Cys 260	c aag gac ac s Lys Asp Th 265	r Cys Pro Pr	a ctc atg ctc o Leu Met L 270	tac aac ccc eu Tyr Asn Pro	816
acc acg tac cag Thr Thr Tyr Glr 275	g atg gat gtg n Met Asp Va 280	aac ccc gag I Asn Pro Gl 28	u Gly Lys Ty	c agc ttt ggt vr Ser Phe Gly	864
gcc acc tgc gtg Ala Thr Cys Val 290	aag aag tgt I Lys Lys Cys 295	ccc cgt aat Pro Arg As 300	tat gtg gtg n Tyr Val Va	aca gat cac Í Thr Asp His	912
ggc tcg tgc gtc Gly Ser Cys Va 305	cga gcc tgt I Arg Ala Cys 310	ggg gcc gac Gly Ala Ası 315	agc tat gag Ser Tyr Gl 320	ı atg gag gaa u Met Glu Glu	960
gac ggc gtc cgc Asp Gly Val Arg 325	J Lys Cys Lys	aag tgc ga Lys Cys Gl 30	a ggg cct tg u Gly Pro Cy 335	c cgc aaa gtg s Arg Lys Val	1008
tgt aac gga ata Cys Asn Gly Ile 340	ggt att ggt Gly Ile Gly (345	Glu Phe Lys	gac tca ctc t Asp Ser Leu 350	cc ata aat 1 I Ser Ile Asn	056
gct acg aat att Ala Thr Asn Ile 355			Thr Ser Ile		104
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cct cct ctg gat o Pro Pro Leu Asp 385	cca cag gaa () Pro Gin Giu 390	tg gat att o Leu Asp Ile 395	tg aaa acc g Leu Lys Th 400	ita aag gaa r Val Lys Glu	1200
atc aca ggg ttt Ile Thr Gly Phe 405	Leu Leu Ile (ng gct tgg co Sin Ala Trp I 10	ct gaa aac a Pro Glu Asn 415	gg acg gac : Arg Thr Asp	1248
ctc cat gcc ttt g Leu His Ala Phe	ag aac cta g Glu Asn Leu	aa atc ata c Glu Ile Ile .	gc.ggc agg a Arg Gly Arg	acc aag caa Thr Lys Gln	1296

gga tta cgc tcc ctc aag gag ata agt gat gga gat gtg ata att tca 1392

445

cat ggt cag ttt tct ctt gca gtc gtc agc ctg aac ata aca tcc ttg 1344 His Gly Gln Phe Ser Leu Ala Val Val Ser Leu Asn Ile Thr Ser Leu

430

425

440

420

																•	
G	iy Leι 450	ı Arg	Ser	Leu 4	Lys 55	Glu	Ile	Ser 46		Gly-	Asp	Val	Ile I	le S	er		
G	ga aa Iy Asr 65	c aaa 1 Lys	Asr	tttg Leu 470	tgc (Cys	tat g Tyr	Ala	aat a Asr 75	aca a n Thi	ata a r Ile	ac to Asn 48	Trp	aa a Lys	aa Lys	ctg Leu	1	440
tt Pl	t ggg ne Gly	/ Thi	tcc g Ser 485	gt c Gly	ag a Gln	Lys	icc a Thr 90	aa Lys	att a Ile 1	ita a Ile S 49	er As	ac a sn A	ga g rg G	igt <u>c</u> Sly C	jaa Slu	1	488
aa As	ac ago sn Se	tgc r Cys 500	s Lys	gcc Ala	Thr	ggc Gly 505	cag Gln	gtc Val	Cys	cat His 510	gcc t Ala l	tg t _eu (gc to Cys	cc co Ser	cc Pro	15	36
ga G	ag gge lu Gly 5:	c tgc Cys 15	tgg Trp	ggc Gly	ccg Pro 520	Glu	ccc Pro	agg Arg	gad Asp 525	Cys	gtc Val	tct t Ser	gc c Cys	gg a Arg	aat Asn	1	584
gt Va	c ago al Ser 530	cga Arg	ggc Gly	Arg	gaa Glu (35	tgc Cys	gtg Val	gac Asp 54	Lys	tgc Cys	aac Asn	ctt (Leu	ctg (Leu	gag Glu	ggt ı Gly	, ,	632
G	ag cca lu Pro 45	agg Arg	Glu	ttt Phe 550	gtg (Val (gag Glu <i>i</i>	Asn	tct Ser 55	gag Glu	tgc a Cys	ata c Ile (56	3In (gc c Cys I	ac c His	ca Pro	. 1 (580
ga Gl	ag tgc lu Cys	Leu	cct o Pro 565	cag g Gln	gcc a Ala I	itg a Met 57	Asn	itc a Ile	cc t Thr	gc a Cys 57	Thr (ga c Gly <i>i</i>	gg g Arg (iga (Gly	cca Pro	1	728
ga As	ac aac sp Asr	tgt Cys 580	s Ile	cag t Gin	Cys .	cc ca Ala l 585	ac ta His T	ac a Tyr :	Ile A	ac gg Isp G	ic co	c ca ro H	c tgo is C	c gto ys V	c 'al	177	6
aa Ly	ng acc s Thr 59	Cys	ccg Pro	gca Ala	gga Gly \ 600	/al N	atg (1et (gga Gly	gaa Glu 605	Asn /	aac Asn	acc Thr I	ctg Leu	gtc Val	tgg Trp	1	824
aa Ly	ng tac 's Tyr 610	gca Ala	gac Asp	gcc Ala (61	Gly⊦⊦	cat (lis V	gtg t al C	tgc ys F 62	lis L	ctg t eu C	gc ca ys H	at co lis P	a aa ro A	ac to sn C	gc Cys	18	72
	c tac ir Tyr 25			aa								:	1887	7			
	2105									-							
	210>	_	,									-					
_	211>						-										:
	212>				. •												
< 2	213>	八(1	nomo	sap	piens)											
_,	1005	ó				•											

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- Leu Ser Leu Gin Arg Met Phe Asn Asn Cys Glu Val Val Leu Gly Asn 50 55 60
- Leu Glu Ile Thr Tyr Val Gln Arg Asn Tyr Asp Leu Ser Phe Leu Lys
 65 70 75 80
- Thr Ile Gin Glu Val Ala Gly Tyr Val Leu Ile Ala Leu Asn Thr Val 85 90 95
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- Ser Met Asp Phe Gln Asn His Leu Gly Ser Cys Gln Lys Cys Asp Pro 180 185 190
- Ser Cys Pro Asn Gly Ser Cys Trp Gly Ala Gly Glu Glu Asn Cys Gln 195 200 205
- Lys Leu Thr Lys Ile Ile Cys Ala Gln Gln Cys Ser Gly Arg Cys Arg 210 215 220
- Gly Lys Ser Pro Ser Asp Cys Cys His Asn Gln Cys Ala Ala Gly Cys 225 230 235 240
- Thr Gly Pro Arg Glu Ser Asp Cys Leu Val Cys Arg Lys Phe Arg Asp 245 250 255
- Glu Ala Thr Cys Lys Asp Thr Cys Pro Pro Leu Met Leu Tyr Asn Pro 260 265 270

- Thr Thr Tyr Gln Met Asp Val Asn Pro Glu Gly Lys Tyr Ser Phe Gly 275 280 285
- Ala Thr Cys Val Lys Lys Cys Pro Arg Asn Tyr Val Val Thr Asp His 290 295 300
- Gly Ser Cys Val Arg Ala Cys Gly Ala Asp Ser Tyr Glu Met Glu Glu 305 310 315 320
- Asp Gly Val Arg Lys Cys Lys Lys Cys Glu Gly Pro Cys Arg Lys Val 325 330 335
- Cys Asn Gly Ile Gly Ile Gly Glu Phe Lys Asp Ser Leu Ser Ile Asn 340 345 350
- Ala Thr Asn Ile Lys His Phe Lys Asn Cys Thr Ser Ile Ser Gly Asp 355 360 365
- Leu His Ile Leu Pro Val Ala Phe Arg Gly Asp Ser Phe Thr His Thr 370 375 380
- Pro Pro Leu Asp Pro Gln Glu Leu Asp Ile Leu Lys Thr Val Lys Glu 385 390 395 400
- Ile Thr Gly Phe Leu Leu Ile Gln Ala Trp Pro Glu Asn Arg Thr Asp 405 410 415
- Leu His Ala Phe Glu Asn Leu Glu Ile Ile Arg Gly Arg Thr Lys Gln 420 425 430
- His Gly Gln Phe Ser Leu Ala Val Val Ser Leu Asn Ile Thr Ser Leu 435 440 445
- Gly Leu Arg Ser Leu Lys Glu Ile Ser Asp Gly Asp Val Ile Ile Ser 450 455 460
- Gly Asn Lys Asn Leu Cys Tyr Ala Asn Thr Ile Asn Trp Lys Lys Leu 465 470 475 480
- Phe Gly Thr Ser Gly Gln Lys Thr Lys Ile Ile Ser Asn Arg Gly Glu 485 490 495
- Asn Ser Cys Lys Ala Thr Gly Gln Val Cys His Ala Leu Cys Ser Pro 500 505 510
- Glu Gly Cys Trp Gly Pro Glu Pro Arg Asp Cys Val Ser Cys Arg Asn

520

525

Val Ser Arg Gly Arg Glu Cys Val Asp Lys Cys Asn Leu Leu Glu Gly 535

Glu Pro Arg Glu Phe Val Glu Asn Ser Glu Cys Ile Gln Cys His Pro 545 555

Glu Cys Leu Pro Gln Ala Met Asn Ile Thr Cys Thr Gly Arg Gly Pro-570 575 565

Asp Asn Cys Ile Gin Cys Ala His Tyr Ile Asp Gly Pro His Cys Val 590 580 585

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Thr Tyr Gly Ser 625

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48

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Leu Glu Ile Thr Tyr Val Gln Arg Asn T	Tyr Asp Leu Ser Phe Leu Lys
65 70 75	80
acc atc cag gag gtg gct ggt tat gtc ct	cc att gcc ctc aac aca gtg 288
Thr Ile Gin Glu Val Ala Gly Tyr Val Le	u Ile Ala Leu Asn Thr Val
85 90	95
gag cga att cct ttg gaa aac ctg cag a	tc atc aga gga aat atg tac 336
Glu Arg Ile Pro Leu Glu Asn Leu Gln I	lle Ile Arg Gly Asn Met Tyr
100 105	110
tac gaa aat tcc tat gcc tta gca gtc tta	tct aac tat gat gca aat 384
Tyr Glu Asn Ser Tyr Ala Leu Ala Val L	eu Ser Asn Tyr Asp Ala Asn
115 120	125
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cat ggc gcc gtg cgg ttc agc aac aac c	ct gcc ctg tgc aac gtg gag 480
His Gly Ala Val Arg Phe Ser Asn Asn F	Pro Ala Leu Cys Asn Val Glu
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Ser Ile Gln Trp Arg Asp Ile Val Ser Se	r Asp Phe Leu Ser Asn Met
165 170	175
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Ser Met Asp Phe Gln Asn His Leu Gly	Ser Cys Gln Lys Cys Asp Pro
180 185	190
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Ser Cys Pro Asn Gly Ser Cys Trp Gly A	Ala Gly Glu Glu Asn Cys Gln
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Gly Lys Ser Pro Ser Asp Cys Cys His A	ssn Gln Cys Ala Ala Gly Cys
225 230 235	240
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Thr Gly Pro Arg Glu Ser Asp Cys Leu V	/al Cys Arg Lys Phe Arg Asp
245 250	255
gaa gcc acg tgc aag gac acc tgc ccc co	ca ctc atg ctc tac aac ccc 816
Glu Ala Thr Cys Lys Asp Thr Cys Pro P	ro Leu Met Leu Tyr Asn Pro
260 265	270
acc acg tac cag atg gat gtg aac ccc ga	g ggc aaa tac agc ttt ggt 864
Fhr Thr Tyr Gln Met Asp Val Asn Pro G	ilu Gly Lys Tyr Ser Phe Gly
275 280 2	85
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295

300

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gct acg aat att aaa cac ttc aaa aac tgc acc tcc atc agt ggc gat 1104 Ala Thr Asn Ile Lys His Phe Lys Asn Cys Thr Ser Ile Ser Gly Asp 355 360 365

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1218

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Gly Thr Ser Asn Lys Leu Thr Gln Leu Gly Thr Phe Glu Asp His Phe 35 40 45

Leu Ser Leu Gln Arg Met Phe Asn Asn Cys Glu Val Val Leu Gly Asn 50 55 60

Leu Glu Ile Thr Tyr Val Gln Arg Asn Tyr Asp Leu Ser Phe Leu Lys 70 75 80

- Thr Ile Gin Giu Val Ala Giy Tyr Val Leu Ile Ala Leu Asn Thr Val 85 90 95
- Glu Arg Ile Pro Leu Glu Asn Leu Gln Ile Ile Arg Gly Asn Met Tyr 100 105 110
- Tyr Glu Asn Ser Tyr Ala Leu Ala Val Leu Ser Asn Tyr Asp Ala Asn 115 120 125
- Lys Thr Gly Leu Lys Glu Leu Pro Met Arg Asn Leu Gln Glu Ile Leu 130 135 140
- His Gly Ala Val Arg Phe Ser Asn Asn Pro Ala Leu Cys Asn Val Glu 145 150 155 160
- Ser Ile Gln Trp Arg Asp Ile Val Ser Ser Asp Phe Leu Ser Asn Met 165 170 175
- Ser Met Asp Phe Gln Asn His Leu Gly Ser Cys Gln Lys Cys Asp Pro 180 185 190
- Ser Cys Pro Asn Gly Ser Cys Trp Gly Ala Gly Glu Glu Asn Cys Gln 195 200 205
- Lys Leu Thr Lys Ile Ile Cys Ala Gln Gln Cys Ser Gly Arg Cys Arg 210 215 220
- Gly Lys Ser Pro Ser Asp Cys Cys His Asn Gln Cys Ala Ala Gly Cys 225 230 235 240
- Thr Gly Pro Arg Glu Ser Asp Cys Leu Val Cys Arg Lys Phe Arg Asp 245 250 255
- Glu Ala Thr Cys Lys Asp Thr Cys Pro Pro Leu Met Leu Tyr Asn Pro 260 265 270
- Thr Thr Tyr Gln Met Asp Val Asn Pro Glu Gly Lys Tyr Ser Phe Gly 275 280 285
- Ala Thr Cys Val Lys Lys Cys Pro Arg Asn Tyr Val Val Thr Asp His 290 295 300
- Gly Ser Cys Val Arg Ala Cys Gly Ala Asp Ser Tyr Glu Met Glu Glu 305 310 315 320
- Asp Gly Val Arg Lys Cys Lys Lys Cys Glu Gly Pro Cys Arg Lys Val 325 330 335

Cys Asn Gly Ile Gly Ile Gly Glu Phe Lys Asp Ser Leu Ser Ile Asn 340 345 350	
Ala Thr Asn Ile Lys His Phe Lys Asn Cys Thr Ser Ile Ser Gly Asp 355 360 365	
Leu His Ile Leu Pro Val Ala Phe Arg Gly Asp Ser Phe Thr His Thr 370 375 380	٠
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otg aca gat cac ggc tcg tgc gtc cga gcc tgt ggg gcc gac agc tat Val Thr Asp His Gly Ser Cys Val Arg Ala Cys Gly Ala Asp Ser Tyr 35 40 45	144
gag atg gag gaa gac ggc gtc cgc aag tgt aag aag tgc gaa ggg cct Glu Met Glu Glu Asp Gly Val Arg Lys Cys Lys Lys Cys Glu Gly Pro 50 55 60	192
gc cgc aaa gtg tgt aac gga ata ggt att ggt gaa ttt aaa gac tca Cys Arg Lys Val Cys Asn Gly Ile Gly Ile Gly Glu Phe Lys Asp Ser 55 70 75 80	240
to too ata aat got acg aat att aaa cac tto aaa aac too acc too a	.00

Leu Ser Ile Ası 85	n Ala Thr As	sn Ile Lys H 90	is Phe Lys A 95	sn Cys Thr Ser	
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acc gta aag ga Thr Val Lys Gli 130			eu Ile Gln A		432
aac agg acg g Asn Arg Thr As 145	ac ctc cat g sp Leu His / 150	cc ttt gag a Ala Phe Glu 155	Asn Leu Glu	atc ata cgc ggc 1 Ile Ile Arg Gly 60	480
agg acc aag co Arg Thr Lys Gl 165	n His Gly G			c agc ctg aac Val Ser Leu Asn	528
	u Gly Leu A			gt gat gga gat Ser Asp Gly Asp	576
				at aca ata aac Asn Thr Ile Asn	624
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	sn Val Ser			gac aag tgc aac I Asp Lys Cys As	816 n
		Arg Glu Phe		tct gag tgc ata n Ser Glu Cys Ile	864
			Ala Met Ası	atc acc tgc aca n Ile Thr Cys Thr	912
			Cys Ala His	tac att gac ggc Tyr Ile Asp Gly 320	960
	al Lys Thr C			gga gaa aac aac Gly Glu Asn Asn	

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gtg ctg ggc tcc ggt gcg ttc ggc acg gtg tat aa Val Leu Gly Ser Gly Ala Phe Gly Thr Val Tyr Ly 450 455 460	g gga ctc tgg atc 1392 ys Gly Leu Trp Ile
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acc ttt gga tcc aag cca tat gac gga atc cct gcc agc gag atc t Thr Phe Gly Ser Lys Pro Tyr Asp Gly Ile Pro Ala Ser Glu Ile S 645 650 655	cc 1968 Ser
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agt cgc cca aag ttc cgt gag ttg atc atc gaa ttc tcc aaa atg gd Ser Arg Pro Lys Phe Arg Glu Leu Ile Ile Glu Phe Ser Lys Met 690 695 700	cc 2112 Ala
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aat ggg ctg caa agc tgt ccc atc aag gaa gac agc ttc ttg cag c Asn Gly Leu Gln Ser Cys Pro Ile Lys Glu Asp Ser Phe Leu Glr 785 790 795 800	cga 2400 i Arg
tac agc tca gac ccc aca ggc gcc ttg act gag gac agc ata gac Tyr Ser Ser Asp Pro Thr Gly Ala Leu Thr Glu Asp Ser Ile Asp 805 810 815	gac 2448 Asp
acc ttc ctc cca gtg cct gaa tac ata aac cag tcc gtt ccc aaa ag Thr Phe Leu Pro Val Pro Glu Tyr Ile Asn Gln Ser Val Pro Lys A 820 825 830	g 2496 Arg
ccc gct ggc tct gtg cag aat cct gtc tat cac aat cag cct ctg aac Pro Ala Gly Ser Val Gln Asn Pro Val Tyr His Asn Gln Pro Leu	: 2544 Asn

840

845

ccc gcg ccc agc aga gac cca cac tac cag gac ccc cac agc act gca 2592
Pro Ala Pro Ser Arg Asp Pro His Tyr Gln Asp Pro His Ser Thr Ala
850 855 860

gtg ggc aac ccc gag tat ctc aac act gtc cag ccc acc tgt gtc aac 2640 Val Gly Asn Pro Glu Tyr Leu Asn Thr Val Gln Pro Thr Cys Val Asn 865 870 875 880

agc aca ttc gac agc cct gcc cac tgg gcc cag aaa ggc agc cac caa 2688 Ser Thr Phe Asp Ser Pro Ala His Trp Ala Gln Lys Gly Ser His Gln 885 890 895

att agc ctg gac aac cct gac tac cag cag gac ttc ttt ccc aag gaa 2736 Ile Ser Leu Asp Asn Pro Asp Tyr Gin Gin Asp Phe Phe Pro Lys Giu 900 905 910

gcc aag cca aat ggc atc ttt aag ggc tcc aca gct gaa aat gca gaa 2784 Ala Lys Pro Asn Gly Ile Phe Lys Gly Ser Thr Ala Glu Asn Ala Glu 915 920 925

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<212> PRT

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Val Thr Asp His Gly Ser Cys Val Arg Ala Cys Gly Ala Asp Ser Tyr 35 40 45

Glu Met Glu Glu Asp Gly Val Arg Lys Cys Lys Lys Cys Glu Gly Pro 50 55 60

Cys Arg Lys Val Cys Asn Gly Ile Gly Ile Gly Glu Phe Lys Asp Ser 65 70 75 80

Leu Ser Ile Asn Ala Thr Asn Ile Lys His Phe Lys Asn Cys Thr Ser 85 90 95

- Ile Ser Gly Asp Leu His Ile Leu Pro Val Ala Phe Arg Gly Asp Ser 100 105 110
- Phe Thr His Thr Pro Pro Leu Asp Pro Gln Glu Leu Asp Ile Leu Lys 115 120 125
- Thr Val Lys Glu Ile Thr Gly Phe Leu Leu Ile Gln Ala Trp Pro Glu 130 135 140
- Asn Arg Thr Asp Leu His Ala Phe Glu Asn Leu Glu Ile Ile Arg Gly 145 150 155 160
- Arg Thr Lys Gln His Gly Gln Phe Ser Leu Ala Val Val Ser Leu Asn 165 170 175
- Ile Thr Ser Leu Gly Leu Arg Ser Leu Lys Glu Ile Ser Asp Gly Asp 180 185 190
- Val Ile Ile Ser Gly Asn Lys Asn Leu Cys Tyr Ala Asn Thr Ile Asn 195 200 205
- Trp Lys Lys Leu Phe Gly Thr Ser Gly Gln Lys Thr Lys Ile Ile Ser 210 215 220
- Asn Arg Gly Glu Asn Ser Cys Lys Ala Thr Gly Gln Val Cys His Ala 225 230 235 240
- Leu Cys Ser Pro Glu Gly Cys Trp Gly Pro Glu Pro Arg Asp Cys Val 245 250 255
- Ser Cys Arg Asn Val Ser Arg Gly Arg Glu Cys Val Asp Lys Cys Asn 260 265 270
- Leu Leu Glu Glu Pro Arg Glu Phe Val Glu Asn Ser Glu Cys Ile 275 280 285
- Gln Cys His Pro Glu Cys Leu Pro Gln Ala Met Asn Ile Thr Cys Thr 290 295 300
- Gly Arg Gly Pro Asp Asn Cys Ile Gln Cys Ala His Tyr Ile Asp Gly 305 310 315 320
- Pro His Cys Val Lys Thr Cys Pro Ala Gly Val Met Gly Glu Asn Asn 325 330 335
- Thr Leu Val Trp Lys Tyr Ala Asp Ala Gly His Val Cys His Leu Cys 340 345 350

- His Pro Asn Cys Thr Tyr Gly Cys Thr Gly Pro Gly Leu Glu Gly Cys 355 360 365
- Pro Thr Asn Gly Pro Lys Ile Pro Ser Ile Ala Thr Gly Met Val Gly 370 375 380
- Ala Leu Leu Leu Leu Val Val Ala Leu Gly Ile Gly Leu Phe Met 385 390 395 400
- Arg Arg Arg His Ile Val Arg Lys Arg Thr Leu Arg Arg Leu Leu Gln 405 410 415
- Glu Arg Glu Leu Val Glu Pro Leu Thr Pro Ser Gly Glu Ala Pro Asn 420 425 430
- Gln Ala Leu Leu Arg Ile Leu Lys Glu Thr Glu Phe Lys Lys Ile Lys 435 440 445
- Vai Leu Gly Ser Gly Ala Phe Gly Thr Val Tyr Lys Gly Leu Trp Ile 450 455 460
- Pro Glu Gly Glu Lys Val Lys Ile Pro Val Ala Ile Lys Glu Leu Arg 465 470 475 480
- Glu Ala Thr Ser Pro Lys Ala Asn Lys Glu Ile Leu Asp Glu Ala Tyr 485 490 495
- Val Met Ala Ser Val Asp Asn Pro His Val Cys Arg Leu Leu Gly Ile 500 505 510
- Cys Leu Thr Ser Thr Val Gln Leu Ile Thr Gln Leu Met Pro Phe Gly 515 520 525
- Cys Leu Leu Asp Tyr Val Arg Glu His Lys Asp Asn Ile Gly Ser Gln 530 535 540
- Tyr Leu Leu Asn Trp Cys Val Gln Ile Ala Lys Gly Met Asn Tyr Leu 545 550 555 560
- Glu Asp Arg Arg Leu Val His Arg Asp Leu Ala Arg Asn Val Leu 565 570 575
- Val Lys Thr Pro Gln His Val Lys Ile Thr Asp Phe Gly Leu Ala Lys 580 585 590
- Leu Leu Gly Ala Glu Glu Lys Glu Tyr His Ala Glu Gly Gly Lys Val 595 600 605

- Pro Ile Lys Trp Met Ala Leu Glu Ser Ile Leu His Arg Ile Tyr Thr 610 615 620
- His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Val Trp Glu Leu Met 625 630 640
- Thr Phe Gly Ser Lys Pro Tyr Asp Gly Ile Pro Ala Ser Glu Ile Ser 645 650 655
- Ser Ile Leu Glu Lys Gly Glu Arg Leu Pro Gln Pro Pro Ile Cys Thr 660 665 670
- Ile Asp Val Tyr Met Ile Met Val Lys Cys Trp Met Ile Asp Ala Asp 675 680 685
- Ser Arg Pro Lys Phe Arg Glu Leu IIe IIe Glu Phe Ser Lys Met Ala 690 695 700
- Arg Asp Pro Gln Arg Tyr Leu Val Ile Gln Gly Asp Glu Arg Met His
 705 710 715 720
- Leu Pro Ser Pro Thr Asp Ser Asn Phe Tyr Arg Ala Leu Met Asp Glu 725 730 735
- Glu Asp Met Asp Asp Val Val Asp Ala Asp Glu Tyr Leu Ile Pro Gln 740 745 750
- Gln Gly Phe Phe Ser Ser Pro Ser Thr Ser Arg Thr Pro Leu Leu Ser 755 760 765
- Ser Leu Ser Ala Thr Ser Asn Asn Ser Thr Val Ala Cys Ile Asp Arg 770 775 780
- Asn Gly Leu Gln Ser Cys Pro Ile Lys Glu Asp Ser Phe Leu Gln Arg 785 790 795 800
- Tyr Ser Ser Asp Pro Thr Gly Ala Leu Thr Glu Asp Ser Ile Asp Asp 805 810 815
- Thr Phe Leu Pro Val Pro Glu Tyr Ile Asn Gln Ser Val Pro Lys Arg 820 825 830
- Pro Ala Gly Ser Val Gln Asn Pro Val Tyr His Asn Gln Pro Leu Asn 835 840 845
- Pro Ala Pro Ser Arg Asp Pro His Tyr Gln Asp Pro His Ser Thr Ala

855

860

Val Gly Asn Pro Glu Tyr Leu Asn Thr Val Gln Pro Thr Cys Val Asn. 865 870 875 880

Ser Thr Phe Asp Ser Pro Ala His Trp Ala Gln Lys Gly Ser His Gln 885 890 895

Ile Ser Leu Asp Asn Pro Asp Tyr Gln Gln Asp Phe Phe Pro Lys Glu 900 905 910

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ggc aca agt aac agg ctc acc caa ctg ggc act ttt gaa gac cac ttt 144 Gly Thr Ser Asn Arg Leu Thr Gln Leu Gly Thr Phe Glu Asp His Phe 35 40 45

ctg agc ctg cag agg atg tac aac aac tgt gaa gtg gtc ctt ggg aac
Leu Ser Leu Gln Arg Met Tyr Asn Asn Cys Glu Val Val Leu Gly Asn
50 55 60

ttg gaa att acc tat gtg caa agg aat tac gac ctt tcc ttc tta aag 240 Leu Glu Ile Thr Tyr Val Gln Arg Asn Tyr Asp Leu Ser Phe Leu Lys 65 70 75 80

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				Glu A				e Arg	gga aat gct Gly Asn Ala		336
tat Tyr	Glu	aac ac Asn Th 15	c tat or Tyr	gcc tt Ala L 120	eu Ala	atc ci i Ile L	tg tcc .eu Se 125	aac t er Asr	at ggg aca a Tyr Gly Thr	ac Asn	384
Arg	act Thr 130	ggg ct Gly Le	u Arg	gaa (Glu i 35	ctg ccc Leu Pro	atg o Met 14	t Arg	ac tta Asn L	cag gaa ato eu Gln Glu II	ctg e Leu	432
att Ile 145	Gly	gct gtg Ala Val	cga Arg F 150	ttc ag Phe S	er Asn	aac c Asn 155	cc ato Pro Il	e Leu	gc aat atg g Cys Asn Mei 160	at t Asp	480
act Thr	atc Ile	cag tgg Gln Trp 165	Arg .	gac a Asp II	tc gtc le Val 170	GIn A	aac gl sn Va	c ttt a I Phe 175	atg agc aac a Met Ser Asn	atg Met	528
tca Ser	atg Mei	gac tta t Asp Le 180	cag eu Gli	า Ser	at ccg His Pro 85	agc a o Ser	agt tg Ser (19	Cys Pr	aaa tgt gat o o Lys Cys As	cca sp Pro	576
agc Ser	Cys	ccc aat Fro As 95	: gga :n Gly	agc to Ser (200	Cys Tr	gga p Gly	gga g Gly (205	ga ga Siy Gi	g gag aac to u Glu Asn Cy	ıc cag /s Gin	624
aaa	tta	acc aa:									
Lys			s Ile				iln Cy		at cgc tgt co His Arg Cys		572
Lys 2 ggc	Leu 210 agg Arg	Thr Ly	s Ile : 2 c agt	Ile Cy 15 gac t	s Ala (gc tgc Cys Cy	GIn G 22 cac a	iln Cy: :0 aac ca	s Ser a tgt Gln Cy		Arg	720
ggc Gly 225 aca	Leu 210 agg Arg	tcc cci Ser Pro	s Ile 2 c agt o Ser 230 a gag g Glu	Ile Cy 15 gac t Asp (ys Ala (gc tgc Cys Cy gac tgl	Gln G 22 cac a s His 235 t ctg	iln Cy: 20 aac ca Asn (gtc tg	s Ser a tgt Gln Cy gc caa	His Arg Cys gct gcg ggg /s Ala Ala Gl	Arg tgt y Cys gat	
ggc Gly 225 aca Thr	Leu 210 agg Arg Gly	tcc ccc Ser Pro ccc cg Pro Arg 245	s Ile 2 c agt o Ser 230 a gag g Glu c aaa	Ile Cy 15 gac ty Asp (gac a Asp T	gc tgc Cys Cy gac tgl Asp Cys 250 acc tgc	cac a s His 235 t ctg s Leu	iln Cy 0 aac ca Asn (gtc tg Val (a tgt Gln Cy Ic caa Cys Gl 255 tc atg	His Arg Cys gct gcg ggg /s Ala Ala Gl 240 aag ttc caa	tgt y Cys gat n Asp	720
ggc Gly 225 aca Thr gag Glu acc	Leu 210 agg Arg Gly gcc Ala	tcc ccc Ser Pro ccc cg Pro Are 245 aca tg Thr Cy: 260 tat cag Tyr Glr	s Ile 2 c agt o Ser 230 a gagg Glu c aaas Lys	gac to Asp (gac to Asp (gac a Asp 7 2	gc tgc Cys Cy gac tgi Asp Cys 250 acc tgc Thr Cys 65	cac as His 235 t ctg s Leu cca s Pro	iln Cy 0 aac ca Asn (gtc tg Val C cca cl Pro L 27 aa gg	a tgt Gln Cy Ic caa Cys Gl 255 tc atg eu Me 0	His Arg Cys gct gcg ggg s Ala Ala Gl 240 aag ttc caa n Lys Phe Gl ctg tac aac	tgt y Cys gat n Asp ccc in Pro	720 768
ggc Gly 225 aca Thr gag Glu acc Thr	Leu 210 agg Arg Gly gcc Ala acc Thr 27 acc	tcc ccc Ser Pro ccc cg Pro Are 245 aca tg Thr Cy: 260 tat cag Tyr Glr 75	s Ile 2 c agt 5 Ser 230 a gag Glu c aaa s Lys atg 9 Met aag 1 Lys	gac to Asp (gac to Ser A gac a Asp (gac a Asp (2 gat gt Asp (280 aag to	gc tgc Cys Cy gac tgt Asp Cys 250 acc tgc Thr Cys 65 cc aac Val Asr	cac as His 235 t ctg s Leu cca s Pro	iln Cy 0 aac ca Asn (gtc tg Val C cca cl Pro L 27 aa gg Glu C 285 aac ta Asn T	a tgt a tgt Gin Cy c caa cys Gi 255 tc atg eu Me 0 g aag Giy Ly	His Arg Cys gct gcg ggg s Ala Ala Gl 240 aag ttc caa n Lys Phe Gl ctg tac aac et Leu Tyr As tac agc ttt c	tgt y Cys gat n Asp ccc n Pro	720 768 816
ggc Gly 225 aca Thr gag Glu acc Thr gcc Ala 2 ggc	ggg Gly gcc Ala acc Thr 27 acc Thr 290 tca Ser	tcc ccc Ser Pro ccc cg Pro Are 245 aca tg Thr Cy: 260 tat cag Tyr Glr 75 tgt gtg Cys Va	s Ile 2 c agt c Ser 230 a gag g Glu c aaa s Lys a Met aag l Lys cga g	gac to Asp (gac to Ser A gac a Asp (gac to 2 gat gt Asp (280 aag to 1 Lys C gcc to 3	gc tgc Cys Cy gac tgi Asp Cys 250 acc tgc Thr Cys 65 cc aac Val Asr gc ccc ys Pro	cac as His 235 to ctg as Proceed as Proceed as Arg 30 cct gas act gas cct gas act gas	iln Cy 0 aac ca Asn (gtc tg Val C cca cl Pro L 27 aa gg Glu C 285 aac ta Asn T	s Ser la tgt Gln Cy lc caa Cys Gl 255 tc atg eu Me 0 g aag Gly Ly: c gtg yr Val ttac g yr Tyi	His Arg Cys gct gcg ggg s Ala Ala Gl 240 aag ttc caa n Lys Phe Gl ctg tac aac et Leu Tyr As tac agc ttt g s Tyr Ser Phe gtg aca gat	tgt y Cys gat n Asp ccc n Pro ggt e Gly cat His	720 768 816 864

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gct aca aa Ala Thr Asi 355	c atc aaa cac n Ile Lys His P 360	'ne Lys Tyr	tgc act gcc a Cys Thr Ala 365	itc agc ggg gac Ile Ser Gly Asp	1104
ctt cac atc Leu His Ile 370	ctg cca gtg g Leu Pro Val A 375	ila Phe Lys	ggg gat tct tto Gly Asp Ser 880	acg cgc act Phe Thr Arg Thr	1152
cct cct cta Pro Pro Let 385	gac cca cga g Asp Pro Arg 390	äa cta gaa Glu Leu Gl 395	u Ile Leu Lys	cc gta aag gaa Thr Val Lys Glu 00	1200
he inr Giy	c ttt ttg ctg at Phe Leu Leu 1 405	t cag gct t lle Gln Ala 410	gg cct gat aa Trp Pro Asp / 415	c tgg act gac Asn Trp Thr Asp	1248
ctc cat gct Leu His Ala 42	Phe Glu Ash	a gaa ata a Leu Glu Ile 125	ata cgt ggc a : Ile Arg Gly A 430	ga aca aag caa Arg Thr Lys Gln	1296
cat ggt cag His Gly Gln 435	ttt tct ttg gcg Phe Ser Leu / 440	Ala Val Val	gc ctg aac atc Gly Leu Asn 445	aca tca ctg 1 Ile Thr Ser Leu	344
ggg ctg cgt Gly Leu Arg 450	tcc ctc aag g Ser Leu Lys 455	Glu Ile Ser	gat ggg gat g Asp Gly Asp 60	itg atc att tct Val Ile Ile Ser	1392
gga aac cga Gly Asn Arg 465	a aat ttg tgc ta Asn Leu Cys 470	ac gca aac Tyr Ala As 475	aca ata aac t n Thr Ile Asn 48	gg aaa aaa ctc Trp Lys Lys Leu 80	1440
Prie Gly Inc	ccc aat cag a Pro Asn Gin I 185	aa acc aaa ys Thr Lys 490	atc atg aac a Ile Met Asn 495	ac aga gct gag Asn Arg Ala Glu	1488
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gaa cca agg Glu Pro Arg 545	gag ttt gtg ga Glu Phe Val G 550	aa aat tct g lu Asn Ser 555	gaa tgc atc ca Glu Cys Ile G 56	in Cys His Pro	1680
Gid CA2 Fén	ccc cag gcc ato Pro Gln Ala M 65	g aac atc a et Asn Ile 570	cc tgt aca gg Thr Cys Thr G 575	c agg ggg cca Gly Arg Gly Pro	1728

Asp Asn Cys Ile Gln Cys Ala His Tyr Ile Asp Gly Pro His Cys Val 580 585 590	
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acc tat gga tgt gct ggg cca ggt ctt caa gga tgt gaa gtg tgg cca Thr Tyr Gly Cys Ala Gly Pro Gly Leu Gln Gly Cys Glu Val Trp Pro 625 630 635 640	1920
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ctc ttc ata gtg gtg gtg gcc ctt ggg att ggc cta ttc atg cga aga 20 Leu Phe Ile Val Val Val Ala Leu Gly Ile Gly Leu Phe Met Arg Arg 660 665 670	016
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acc tcc act gtc cag ctc att aca cag ctc atg ccc tac ggt tgc ctc 24 Thr Ser Thr Val Gln Leu Ile Thr Gln Leu Met Pro Tyr Gly Cys Leu 785 790 795 800	00
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ctc aac tgg tgt gtg cag att gca aag ggc atg aac tac ctg gaa gat Leu Asn Trp Cys Val Gln Ile Ala Lys Gly Met Asn Tyr Leu Glu Asp 820 825 830	2496 _.

AIY AI	t ttg gtg g Leu Va 335	cac cgt (I His Arg 84	Asp Lei	gca gcc u Ala Ala 84	a Arg A	t gta ctg g sn Val Leu	itg aag Val Ly:	2544 s
aca cc Thr Pro 850	פות וווט ט	gtc aag Val Lys 1 855	atc aca lle Thr /	gat ttt Asp Phe 860	ggg ctg Gly Lei	gcc aaa c ı Ala Lys I	tg ctt .eu Leu	2592
ggt gd Gly Ala 865	t gaa gag a Glu Glu	j aaa gaa Lys Glu 870	lyr His	gcc gag Ala Glu 875	g ggg g Gly Gly	gc aaa gto Lys Val P 880	cct ato	2640
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Giy Sei	aag cct Lys Pro 15	tat gat g Tyr Asp 920	Gly He I	cca gca Pro Ala ! 92!	Ser Asp	atc tca to Ile Ser Se	c atc er Ile	2784
cta gag Leu Glu 930	aaa gga Lys Gly	gaa cgc Glu Arg 935	ctt cca Leu Pro	cag cca Gln Pro 940	cct atc Pro Ile	tgc acc at Cys Thr I	tc gat le Asp	2832
gtc tac Val Tyr 945	Met He L	itg gtc aa Met Val L 950	ys Cys T	gg atg a Frp Met 55	Ile Asp	gct gat ago Ala Asp S 960	c cgc er Arg	2880
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cca cag Pro Gln	cgc tac o Arg Tyr I 980	ren val I	c cag gg le Gln G 85	ily Asp (aa aga Slu Arg 190	atg cat tto Met His Lo	g cca eu Pro	2976
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tct ctg a Ser Leu 1040	agt gca a Ser Ala	ct agc aa Thr Ser A 1045	ıc aatt Isn Asn	cc act g Ser Th 1050	r Val Al	tgc att aal a Cys Ile	t 31 Asn	59
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gac gca ttc ctc cct gta cct gaa tat gta aac caa tct gtt ccc Asp Ala Phe Leu Pro Val Pro Glu Tyr Val Asn Gln Ser Val Pro 1090 1095 aag agg cca gca ggc tct gtg cag aac cct gtc tat cac aat cag 3339 Lys Arg Pro Ala Gly Ser Val Gln Asn Pro Val Tyr His Asn Gln 1100 1105 1110 ccc ctg cat cca gct cct gga aga gac ctg cat tat caa aat ccc 3384 Pro Leu His Pro Ala Pro Gly Arg Asp Leu His Tyr Gln Asn Pro 1115 1120 1125 cac age aat gca gtg ggc aac cct gag tat ctc aac act gcc cag 3429 His Ser Asn Ala Val Gly Asn Pro Glu Tyr Leu Asn Thr Ala Gln 1130 1135 1140 cct acc tgt ctc agt agt ggg ttt aac agc cct gca ctc tgg atc Pro Thr Cys Leu Ser Ser Gly Phe Asn Ser Pro Ala Leu Trp Ile 1150 1155 cag aaa ggc agt cac caa atg agc cta gac aac cct gac tac cag 3519 Gin Lys Gly Ser His Gin Met Ser Leu Asp Asn Pro Asp Tyr Gin 1165 1170 cag gac ttc ttc ccc aag gaa acc aag cca aat ggc ata ttt aag 3564 Gln Asp Phe Phe Pro Lys Glu Thr Lys Pro Asn Gly Ile Phe Lys 1175 1180 ggc ccc aca gct gaa aat gca gag tac cta cgg gtg gca cct cca 3609 Gly Pro Thr Ala Glu Asn Ala Glu Tyr Leu Arg Val Ala Pro Pro 1195 1200 agc agc gag ttt att gga gca tga 3633 Ser Ser Glu Phe Ile Gly Ala 1205 1210 <210> 14 <211> 1210 <212> PRT <213> 小鼠(Mus musculus) <400> 14 Met Arg Pro Ser Gly Thr Ala Arg Thr Thr Leu Leu Val Leu Leu Thr 10 15 Ala Leu Cys Ala Ala Gly Gly Ala Leu Glu Glu Lys Lys Val Cys Gln 25 30 Gly Thr Ser Asn Arg Leu Thr Gln Leu Gly Thr Phe Glu Asp His Phe 35

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Tyr Glu Asn Thr Tyr Ala Leu Ala Ile Leu Ser Asn Tyr Gly Thr Asn 115 120 125

Arg Thr Gly Leu Arg Glu Leu Pro Met Arg Asn Leu Gln Glu Ile Leu 130 135 140

Ile Gly Ala Val Arg Phe Ser Asn Asn Pro Ile Leu Cys Asn Met Asp 145 150 155 160

Thr Ile Gln Trp Arg Asp Ile Val Gln Asn Val Phe Met Ser Asn Met 165 170 175

Ser Met Asp Leu Gln Ser His Pro Ser Ser Cys Pro Lys Cys Asp Pro 180 185 190

Ser Cys Pro Asn Gly Ser Cys Trp Gly Gly Gly Glu Glu Asn Cys Gln 195 200 205

Lys Leu Thr Lys Ile Ile Cys Ala Gln Gln Cys Ser His Arg Cys Arg 210 215 220

Gly Arg Ser Pro Ser Asp Cys Cys His Asn Gln Cys Ala Ala Gly Cys 225 230 235 240

Thr Gly Pro Arg Glu Ser Asp Cys Leu Val Cys Gln Lys Phe Gln Asp 245 250 255

Glu Ala Thr Cys Lys Asp Thr Cys Pro Pro Leu Met Leu Tyr Asn Pro 260 265 270

Thr Thr Tyr Gln Met Asp Val Asn Pro Glu Gly Lys Tyr Ser Phe Gly 275 280 285

Ala Thr Cys Val Lys Lys Cys Pro Arg Asn Tyr Val Val Thr Asp His 290 295 300

- Gly Ser Cys Val Arg Ala Cys Gly Pro Asp Tyr Tyr Glu Val Glu Glu 305 310 315 320
- Asp Gly Ile Arg Lys Cys Lys Lys Cys Asp Gly Pro Cys Arg Lys Val 325 330 335
- Cys Asn Gly Ile Gly Ile Gly Glu Phe Lys Asp Thr Leu Ser Ile Asn 340 345 350
- Ala Thr Asn Ile Lys His Phe Lys Tyr Cys Thr Ala Ile Ser Gly Asp 355 360 365
- Leu His Ile Leu Pro Val Ala Phe Lys Gly Asp Ser Phe Thr Arg Thr 370 375 380
- Pro Pro Leu Asp Pro Arg Glu Leu Glu Ile Leu Lys Thr Val Lys Glu 385 390 395 400
- Ile Thr Gly Phe Leu Leu Ile Gln Ala Trp Pro Asp Asn Trp Thr Asp 405 410 415
- Leu His Ala Phe Glu Asn Leu Glu Ile Ile Arg Gly Arg Thr Lys Gln 420 425 430
- His Gly Gln Phe Ser Leu Ala Val Val Gly Leu Asn Ile Thr Ser Leu 435 440 445
- Gly Leu Arg Ser Leu Lys Glu Ile Ser Asp Gly Asp Val Ile Ile Ser 450 455 460
- Gly Asn Arg Asn Leu Cys Tyr Ala Asn Thr Ile Asn Trp Lys Lys Leu 465 470 475 480
- Phe Gly Thr Pro Asn Gln Lys Thr Lys Ile Met Asn Asn Arg Ala Glu 485 490 495
- Lys Asp Cys Lys Ala Val Asn His Val Cys Asn Pro Leu Cys Ser Ser 500 505 510
- Glu Gly Cys Trp Gly Pro Glu Pro Arg Asp Cys Val Ser Cys Gln Asn 515 520 525
- Val Ser Arg Gly Arg Glu Cys Val Glu Lys Cys Asn Ile Leu Glu Gly 530 535 540
- Glu Pro Arg Glu Phe Val Glu Asn Ser Glu Cys Ile Gln Cys His Pro 545 550 555 560

- Glu Cys Leu Pro Gln Ala Met Asn Ile Thr Cys Thr Gly Arg Gly Pro 565 570 575
- Asp Asn Cys Ile Gln Cys Ala His Tyr Ile Asp Gly Pro His Cys Val 580 585 590
- Lys Thr Cys Pro Ala Gly Ile Met Gly Glu Asn Asn Thr Leu Val Trp $595 \hspace{1cm} 600 \hspace{1cm} 605$
- Lys Tyr Ala Asp Ala Asn Asn Val Cys His Leu Cys His Ala Asn Cys 610 615 620
- Thr Tyr Gly Cys Ala Gly Pro Gly Leu Gln Gly Cys Glu Val Trp Pro 625 630 635 640
- Ser Gly Pro Lys Ile Pro Ser Ile Ala Thr Gly Ile Val Gly Gly Leu 645 650 655
- Leu Phe Ile Val Val Val Ala Leu Gly Ile Gly Leu Phe Met Arg Arg 660 665 670
- Arg His Ile Val Arg Lys Arg Thr Leu Arg Arg Leu Leu Gln Glu Arg 675 680 685
- Glu Leu Val Glu Pro Leu Thr Pro Ser Gly Glu Ala Pro Asn Gln Ala 690 695 700
- His Leu Arg Ile Leu Lys Glu Thr Glu Phe Lys Lys Ile Lys Val Leu 705 710 715 720
- Gly Ser Gly Ala Phe Gly Thr Val Tyr Lys Gly Leu Trp Ile Pro Glu 725 730 735
- Gly Glu Lys Val Lys Ile Pro Val Ala Ile Lys Glu Leu Arg Glu Ala 740 745 750
- Thr Ser Pro Lys Ala Asn Lys Glu Ile Leu Asp Glu Ala Tyr Val Met 755 760 765
- Ala Ser Val Asp Asn Pro His Val Cys Arg Leu Leu Gly Ile Cys Leu 770 775 780
- Thr Ser Thr Val Gln Leu Ile Thr Gln Leu Met Pro Tyr Gly Cys Leu 785 790 795 800
- Leu Asp Tyr Val Arg Glu His Lys Asp Asn Ile Gly Ser Gln Tyr Leu 805 810 815

- Leu Asn Trp Cys Val Gin Ile Ala Lys Giy Met Asn Tyr Leu Giu Asp 820 825 830
- Arg Arg Leu Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Lys 835 840 845
- Thr Pro Gln His Val Lys Ile Thr Asp Phe Gly Leu Ala Lys Leu Leu 850 855 860
- Gly Ala Glu Glu Lys Glu Tyr His Ala Glu Gly Gly Lys Val Pro Ile 865 870 875 880
- Lys Trp Met Ala Leu Glu Ser Ile Leu His Arg Ile Tyr Thr His Gln 885 890 895
- Ser Asp Val Trp Ser Tyr Gly Val Thr Val Trp Glu Leu Met Thr Phe 900 905 910
- Gly Ser Lys Pro Tyr Asp Gly Ile Pro Ala Ser Asp Ile Ser Ser Ile 915 920 925
- Leu Glu Lys Gly Glu Arg Leu Pro Gln Pro Pro Ile Cys Thr Ile Asp 930 935 940
- Val Tyr Met Ile Met Val Lys Cys Trp Met Ile Asp Ala Asp Ser Arg 945 950 955 960
- Pro Lys Phe Arg Glu Leu Ile Leu Glu Phe Ser Lys Met Ala Arg Asp 965 970 975
- Pro Gln Arg Tyr Leu Val Ile Gln Gly Asp Glu Arg Met His Leu Pro 980 985 990
- Ser Pro Thr Asp Ser Asn Phe Tyr Arg Ala Leu Met Asp Glu Glu Asp 995 1000 1005
- Met Glu Asp Val Val Asp Ala Asp Glu Tyr Leu Thr Pro Gin Gln 1010 1015 1020
- Gly Phe Phe Asn Ser Pro Ser Thr Ser Arg Thr Pro Leu Leu Ser 1025 1030 1035
- Ser Leu Ser Ala Thr Ser Asn Asn Ser Thr Val Ala Cys Ile Asn 1040 1045 1050
- Arg Asn Gly Ser Cys Arg Val Lys Glu Asp Ala Phe Leu Gln Arg

1060

1065

Tyr Ser Ser Asp Pro Thr Gly Ala Val Thr Glu Asp Asn Ile Asp 1070 1075 1080

Asp Ala Phe Leu Pro Val Pro Glu Tyr Val Asn Gln Ser Val Pro 1085 1090 1095

Lys Arg Pro Ala Gly Ser Val Gln Asn Pro Val Tyr His Asn Gln 1100 1105 1110

Pro Leu His Pro Ala Pro Gly Arg Asp Leu His Tyr Gln Asn Pro 1115 1120 1125

His Ser Asn Ala Val Gly Asn Pro Glu Tyr Leu Asn Thr Ala Gln 1130 1135 1140

Pro Thr Cys Leu Ser Ser Gly Phe Asn Ser Pro Ala Leu Trp Ile 1145 1150 1155

Gln Lys Gly Ser His Gln Met Ser Leu Asp Asn Pro Asp Tyr Gln 1160 1165 1170

Gln Asp Phe Phe Pro Lys Glu Thr Lys Pro Asn Gly Ile Phe Lys 1175 1180 1185

Gly Pro Thr Ala Glu Asn Ala Glu Tyr Leu Arg Val Ala Pro Pro 1190 1195 1200

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<210> 15

<211> 1932

<212> DNA

<213> 小鼠(Mus musculus)

<220>

<221> CDS

<222> (1)..(1929)

<223>

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gcg ctc tgc gcc gca ggt ggg gcg ttg gag gaa aag aaa gtc tgc ca Ala Leu Cys Ala Ala Giy Giy Ala Leu Giu Giu Lys Lys Val Cys Gir 20 25 30	a 96 1
ggc aca agt aac agg ctc acc caa ctg ggc act ttt gaa gac cac ttt Gly Thr Ser Asn Arg Leu Thr Gln Leu Gly Thr Phe Glu Asp His P 35 40 45	144 he
ctg agc ctg cag agg atg tac aac aac tgt gaa gtg gtc ctt ggg aac Leu Ser Leu Gln Arg Met Tyr Asn Asn Cys Glu Val Val Leu Gly A 50 55 60	192 .sn
ttg gaa att acc tat gtg caa agg aat tac gac ctt tcc ttc tta aag Leu Glu Ile Thr Tyr Val Gln Arg Asn Tyr Asp Leu Ser Phe Leu Ly 65 70 75 80	240 s
acc atc cag gag gtg gcc ggc tat gtc ctc att gcc ctc aac acc gtg Thr Ile Gln Glu Val Ala Gly Tyr Val Leu Ile Ala Leu Asn Thr Val 85 90 95	288
gag aga atc cct ttg gag aac ctg cag atc atc agg gga aat gct ctt Glu Arg Ile Pro Leu Glu Asn Leu Gln Ile Ile Arg Gly Asn Ala Leu 100 105 110	336
tat gaa aac acc tat gcc tta gcc atc ctg tcc aac tat ggg aca aac Tyr Glu Asn Thr Tyr Ala Leu Ala Ile Leu Ser Asn Tyr Gly Thr Asn 115 120 125	384
aga act ggg ctt agg gaa ctg ccc atg cgg aac tta cag gaa atc ctg Arg Thr Gly Leu Arg Glu Leu Pro Met Arg Asn Leu Gln Glu Ile Le 130 135 140	432 u
att ggt gct gtg cga ttc agc aac aac ccc atc ctc tgc aat atg gat Ile Gly Ala Val Arg Phe Ser Asn Asn Pro Ile Leu Cys Asn Met Asp 145 150 155 160	480
act atc cag tgg agg gac atc gtc caa aac gtc ttt atg agc aac atg Thr Ile Gln Trp Arg Asp Ile Val Gln Asn Val Phe Met Ser Asn Met 165 170 175	528
tca atg gac tta cag agc cat ccg agc agt tgc ccc aaa tgt gat cca Ser Met Asp Leu Gln Ser His Pro Ser Ser Cys Pro Lys Cys Asp Pr 180 185 190	576 o
agc tgt ccc aat gga agc tgc tgg gga gga gga gag gag aac tgc ca Ser Cys Pro Asn Gly Ser Cys Trp Gly Gly Gly Glu Glu Asn Cys Gl 195 200 205	g 624 n
aaa ttg acc aaa atc atc tgt gcc cag caa tgt tcc cat cgc tgt cgt Lys Leu Thr Lys Ile Ile Cys Ala Gln Gln Cys Ser His Arg Cys Arg 210 215 220	672
ggc agg tcc ccc agt gac tgc tgc cac aac caa tgt gct gcg ggg tgt Gly Arg Ser Pro Ser Asp Cys Cys His Asn Gln Cys Ala Ala Gly Cys 225 230 235 240	7.20 s
aca ggg ccc cga gag agt gac tgt ctg gtc tgc caa aag ttc caa gat Thr Gly Pro Arg Glu Ser Asp Cys Leu Val Cys Gln Lys Phe Gln As 52	768 p

245	250	255

gag gcc aca tgc aaa gac acc tgc cca cca ctc atg ctg tac aac ccc 816
Glu Ala Thr Cys Lys Asp Thr Cys Pro Pro Leu Met Leu Tyr Asn Pro
260 265 270

acc acc tat cag atg gat gtc aac cct gaa ggg aag tac agc ttt ggt
Thr Thr Tyr Gln Met Asp Val Asn Pro Glu Gly Lys Tyr Ser Phe Gly
275 280 285

gcc acc tgt gtg aag aag tgc ccc cga aac tac gtg gtg aca gat cat
Ala Thr Cys Val Lys Lys Cys Pro Arg Asn Tyr Val Val Thr Asp His
290
295
300

ggc tca tgt gtc cga gcc tgt ggg cct gac tac tac gaa gtg gaa gaa 960 Gly Ser Cys Val Arg Ala Cys Gly Pro Asp Tyr Tyr Glu Val Glu Glu 305 310 315 320

gat ggc atc cgc aag tgt aaa aaa tgt gat ggg ccc tgt cgc aaa gtt 1008 Asp Gly Ile Arg Lys Cys Lys Lys Cys Asp Gly Pro Cys Arg Lys Val 325 330 335

tgt aat ggc ata ggc att ggt gaa ttt aaa gac aca ctc tcc ata aat

Cys Asn Gly Ile Gly Ile Gly Glu Phe Lys Asp Thr Leu Ser Ile Asn

340

345

350

gct aca aac atc aaa cac ttc aaa tac tgc act gcc atc agc ggg gac
Ala Thr Asn Ile Lys His Phe Lys Tyr Cys Thr Ala Ile Ser Gly Asp
355
360
365

ctt cac atc ctg cca gtg gcc ttt aag ggg gat tct ttc acg cgc act 1152 Leu His Ile Leu Pro Val Ala Phe Lys Gly Asp Ser Phe Thr Arg Thr 370 375 380

cct cct cta gac cca cga gaa cta gaa att cta aaa acc gta aag gaa Pro Pro Leu Asp Pro Arg Glu Leu Glu Ile Leu Lys Thr Val Lys Glu 385 390 395 400

ata aca ggc ttt ttg ctg att cag gct tgg cct gat aac tgg act gac 1248
Ile Thr Gly Phe Leu Leu Ile Gln Ala Trp Pro Asp Asn Trp Thr Asp
405 410 415

ctc cat gct ttc gag aac cta gaa ata ata cgt ggc aga aca aag caa 1296 Leu His Ala Phe Glu Asn Leu Glu Ile Ile Arg Gly Arg Thr Lys Gln 420 425 430

cat ggt cag ttt tct ttg gcg gtc gtt ggc ctg aac atc aca tca ctg 1344 His Gly Gln Phe Ser Leu Ala Val Val Gly Leu Asn Ile Thr Ser Leu 435 440 445

ggg ctg cgt tcc ctc aag gag atc agt gat ggg gat gtg atc att tct 1392 Gly Leu Arg Ser Leu Lys Glu Ile Ser Asp Gly Asp Val Ile Ile Ser 450 455 460

gga aac cga aat ttg tgc tac gca aac aca ata aac tgg aaa aaa ctc 1440 Gly Asn Arg Asn Leu Cys Tyr Ala Asn Thr Ile Asn Trp Lys Lys Leu 465 470 475 480

ttc ggg aca ccc aat cag aaa acc aaa atc atg aac aac aga gct gag
Phe Gly Thr Pro Asn Gln Lys Thr Lys Ile Met Asn Asn Arg Ala Glu
485
490
495

aaa gac tgc aag gcc gtg aac cac gtc tgc aat cct tta tgc tcc tcg 1536

Lys Asp Cys Lys Ala Val Asn His Val Cys Asn Pro Leu Cys Ser Ser 500 505 510

gaa ggc tgc tgg ggc cct gag ccc agg gac tgt gtc tcc tgc cag aat 1584 Glu Gly Cys Trp Gly Pro Glu Pro Arg Asp Cys Val Ser Cys Gln Asn 515 520 525

gtg agc aga ggc agg gag tgc gtg gag aaa tgc aac atc ctg gag ggg 1632 Val Ser Arg Gly Arg Glu Cys Val Glu Lys Cys Asn Ile Leu Glu Gly 530 535 540

gaa cca agg gag ttt gtg gaa aat tct gaa tgc atc cag tgc cat cca 1680 Glu Pro Arg Glu Phe Val Glu Asn Ser Glu Cys Ile Gln Cys His Pro 545 550 555 560

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gac aac tgc atc cag tgt gcc cac tac att gat ggc cca cac tgt gtc 1776 Asp Asn Cys Ile Gln Cys Ala His Tyr Ile Asp Gly Pro His Cys Val 580 585 590

aag acc tgc cca gct ggc atc atg gga gag aac aac act ctg gtc tgg
Lys Thr Cys Pro Ala Gly Ile Met Gly Glu Asn Asn Thr Leu Val Trp
595 600 605

aag tat gca gat gcc aat aat gtc tgc cac cta tgc cac gcc aac tgt

Lys Tyr Ala Asp Ala Asn Asn Val Cys His Leu Cys His Ala Asn Cys

610

615

620

acc tat gga gta atg gtt cct gaa atg ttg ctc caa agt atc att tta 1920 Thr Tyr Gly Val Met Val Pro Glu Met Leu Leu Gln Ser Ile Ile Leu 625 630 635 640

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<210> 16

. <211> 643

<212> PRT

<213> 小鼠(Mus musculus)

<400> 16

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Ala Leu Cys Ala Ala Gly Gly Ala Leu Glu Glu Lys Lys Val Cys Gln 20 25 30

Gly Thr Ser Asn Arg Leu Thr Gln Leu Gly Thr Phe Glu Asp His Phe 35 40 45

- Leu Ser Leu Gln Arg Met Tyr Asn Asn Cys Glu Val Val Leu Gly Asn
 50
 55
 60
- Leu Glu Ile Thr Tyr Val Gln Arg Asn Tyr Asp Leu Ser Phe Leu Lys 65 70 75 80
- Thr Ile Gin Glu Val Ala Gly Tyr Val Leu Ile Ala Leu Asn Thr Val 85 90 95
- Glu Arg Ile Pro Leu Glu Asn Leu Gln Ile Ile Arg Gly Asn Ala Leu 100 105 110
- Tyr Glu Asn Thr Tyr Ala Leu Ala Ile Leu Ser Asn Tyr Gly Thr Asn 115 120 125
- Arg Thr Gly Leu Arg Glu Leu Pro Met Arg Asn Leu Gln Glu Ile Leu 130 135 140
- Ile Gly Ala Val Arg Phe Ser Asn Asn Pro Ile Leu Cys Asn Met Asp 145 150 155 160
- Thr Ile Gln Trp Arg Asp Ile Val Gln Asn Val Phe Met Ser Asn Met 165 170 175
- Ser Met Asp Leu Gln Ser His Pro Ser Ser Cys Pro Lys Cys Asp Pro 180 185 190
- Ser Cys Pro Asn Gly Ser Cys Trp Gly Gly Gly Glu Glu Asn Cys Gln 195 200 205
- Lys Leu Thr Lys Ile Ile Cys Ala Gln Gln Cys Ser His Arg Cys Arg 210 215 220
- Gly Arg Ser Pro Ser Asp Cys Cys His Asn Gln Cys Ala Ala Gly Cys 225 230 235 240
- Thr Gly Pro Arg Glu Ser Asp Cys Leu Val Cys Gln Lys Phe Gln Asp 245 250 255
- Glu Ala Thr Cys Lys Asp Thr Cys Pro Pro Leu Met Leu Tyr Asn Pro 260 265 270
- Thr Thr Tyr Gln Met Asp Val Asn Pro Glu Gly Lys Tyr Ser Phe Gly 275 280 285
- Ala Thr Cys Val Lys Lys Cys Pro Arg Asn Tyr Val Val Thr Asp His 290 295 300

- Gly Ser Cys Val Arg Ala Cys Gly Pro Asp Tyr Tyr Glu Val Glu Glu 305 310 315 320
- Asp Gly Ile Arg Lys Cys Lys Cys Asp Gly Pro Cys Arg Lys Val 325 330 335
- Cys Asn Gly Ile Gly Ile Gly Glu Phe Lys Asp Thr Leu Ser Ile Asn 340 345 350
- Ala Thr Asn Ile Lys His Phe Lys Tyr Cys Thr Ala Ile Ser Gly Asp 355 360 365
- Leu His Ile Leu Pro Val Ala Phe Lys Gly Asp Ser Phe Thr Arg Thr 370 375 380
- Pro Pro Leu Asp Pro Arg Glu Leu Glu Ile Leu Lys Thr Val Lys Glu 385 390 395 400
- Ile Thr Gly Phe Leu Leu Ile Gln Ala Trp Pro Asp Asn Trp Thr Asp 405 410 415
- Leu His Ala Phe Glu Asn Leu Glu Ile Ile Arg Gly Arg Thr Lys Gln 420 425 430
- His Gly Gin Phe Ser Leu Ala Val Val Gly Leu Asn Ile Thr Ser Leu 435 440 445
- Gly Leu Arg Ser Leu Lys Glu Ile Ser Asp Gly Asp Val Ile Ile Ser 450 455 460
- Gly Asn Arg Asn Leu Cys Tyr Ala Asn Thr Ile Asn Trp Lys Lys Leu 465 470 475 480
- Phe Gly Thr Pro Asn Gln Lys Thr Lys Ile Met Asn Asn Arg Ala Glu 485 490 495
- Lys Asp Cys Lys Ala Val Asn His Val Cys Asn Pro Leu Cys Ser Ser 500 505 510
- Glu Gly Cys Trp Gly Pro Glu Pro Arg Asp Cys Val Ser Cys Gln Asn 515 520 525
- Val Ser Arg Gly Arg Glu Cys Val Glu Lys Cys Asn Ile Leu Glu Gly 530 535 540
- Glu Pro Arg Glu Phe Val Glu Asn Ser Glu Cys Ile Gln Cys His Pro 56

550 ...

555

560

Glu Cys Leu Pro Gln Ala Met Asn Ile Thr Cys Thr Gly Arg Gly Pro 565 570 575

Asp Asn Cys Ile Gln Cys Ala His Tyr Ile Asp Gly Pro His Cys Val 580 585 590

Lys Thr Cys Pro Ala Gly Ile Met Gly Glu Asn Asn Thr Leu Val Trp 595 600 605

Lys Tyr Ala Asp Ala Asn Asn Val Cys His Leu Cys His Ala Asn Cys 610 615 620

Thr Tyr Gly Val Met Val Pro Glu Met Leu Leu Gln Ser Ile Ile Leu 625 630 635 640

Lys Pro Ile

<210> 17

<211> 1968

<212> DNA

<213> 小鼠(Mus musculus)

<220>

<221> CDS

<222> (1)..(1965)

<223>

<400> 17

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Ala Leu Cys Ala Ala Gly Gly Ala Leu Glu Glu Lys Lys Vai Cys Gln
20 25 30

ggc aca agt aac agg ctc acc caa ctg ggc act ttt gaa gac cac ttt 144 Gly Thr Ser Asn Arg Leu Thr Gln Leu Gly Thr Phe Glu Asp His Phe

ctg agc ctg cag agg atg tac aac agc tgt gaa gtg gtc ctt ggg aac 192 Leu Ser Leu Gln Arg Met Tyr Asn Asn Cys Glu Val Val Leu Gly Asn 50 55 60

Le 65	eu Gl	u Ile	Thr Tyr	gtg caa 'Val Gln	agg aat Arg Ası 75	tac ga n Tyr A	c ctt tcc sp Leu 9 80	ttc tta aag Ser Phe Leu Ly	240 's
ac Th	c ato	Gln (gag gte Glu Val 35	Ala Gly	c tat gto Tyr Val 90	Leu Ile	t gcc cto Ala Leu 95	aac acc gtg Asn Thr Val	288
ga Gl	ig ag u Ar	a atc g Ile i 100	Pro Leu	gag aad Glu Asr 10!	ı Leu Gl	g atc at n Ile Ile 11	e Arg Gl	ga aat gct ctt y Asn Ala Leu	336
ta: Ty	r Gli	a aac ı Asn 15	acc tat Thr Ty	gcc tta r Ala Let 120	gcc atc ı Ala İle	ctg tcc Leu Se 125	aac tat er Asn T	ggg aca aac yr Gly Thr Asn	384 1
ag Ar	a ac g Th 130	t ggg r Gly	Leu Ar	g gaa cto g Glu Le 135	u Pro M	g cgg a et Arg / 140	ac tta ca Asn Leu	ag gaa atc ctg Gln Glu Ile Le	432 eu
ati Ile 14	Gly	gct g Ala V	tg cga 'al Arg 150	Phe Ser	aac aac Asn Ası 155	n Pro II	ctc tgc e Leu Cy 16	aat atg gat ys Asn Met Asp 0	480 p
ac Th	t atc r Ile	Gln T	gg agg rp Arg 65	Asp Ile	gtc caa Val Gln 170	aac gt Asn Va	c ttt ato I Phe Mo 175	g agc aac atg et Ser Asn Met	528 t
tca Se	a atg er Me	gac t t Asp 180	Leu Gl	agc cat In Ser Hi 185	s Pro Se	agt tg er Ser (19	Cys Pro	a tgt gat cca Lys Cys Asp Pi	576 ro
ag Se	r Cy	ccc a s Pro 95	at gga Asn Gl	agc tgc y Ser Cy 200	tgg gga s Trp G	a gga g ly Gly 0 205	ga gag Gly Glu (gag aac tgc ca Glu Asn Cys Gl	ig 624 In
aa Ly:	a ttg s Lei 210	acc a	aaa atc	atc tgt	gcc cag	caa tot	tcc cat		
99		1 1111	Lys Ile	Ile Cys 215		Gln Cy: 20	s Ser Hi	s Arg Cys Arg	672
Gly 22	c ag y Arg	g tcc	Lys Ile 2 ccc agt	215 gac tgc Asp Cy	tgc cac	GIn Cy: 220 aac ca is Asn (s Ser Hi a tgt gc	s Arg Cys Arg t gcg ggg tgt Ala Ala Gly Cy	720
Gly 22 aca	c ag y Arg 5 a gg:	g tcc Ser ccc Pro /	Lys Ile 2 ccc agt Pro Ser 230 cga ga	215 gac tgc Asp Cy g agt ga Ser Asp	tgc cac s Cys Hi 235 c tgt cto	GIn Cys 220 aac ca is Asn (s Ser Hi a tgt gc Gln Cys 240 c caa aa	s Arg Cys Arg t gcg ggg tgt Ala Ala Gly Cy	720 s 768
Gly 22 aca Th	c ag y Arg 5 a ggg r Gly	g tcc (Ser) g ccc (Pro / 20	Lys Ile 2 ccc agt Pro Ser 230 cga ga Arg Glu 45 tgc aaa	gac tgc r Asp Cy g agt ga s Ser Asp a gac acc	tgc cac s Cys Hi 235 c tgt ctc Cys Le 250 c tgc cca c Cys Pr	Gln Cy: 220 aac ca is Asn (g gtc tg u Val C	a tgt go Gln Cys 240 c caa aa ys Gln I 255 c atg ct eu Met I	s Arg Cys Arg t gcg ggg tgt Ala Ala Gly Cy 0	720 s 768 sp 816
aca Th ga Glu	c ag y Arg 5 a ggg r Gly g gcg J Ala	g tcc (g ccc (Pro / 2 c aca Thr (260)	ccc agt Pro Ser 230 cga gar Arg Glu 45 tgc aaa	gac tgc r Asp Cy g agt ga s Ser Asp a gac acc Asp Thi 265 gat gtc	tgc cac s Cys Hi 235 c tgt ctc c Cys Le 250 c tgc ccc c Cys Pr	Gln Cy: 220 aac ca is Asn (g gtc tg u Val C a cca ct o Pro L 27	a tgt go Gln Cys 240 c caa aa ys Gln I 255 c atg ct eu Met I 0	t gcg ggg tgt Ala Ala Gly Cy: 0 ag ttc caa gat Lys Phe Gln As	720 s 768 sp 816 o 864
aca Th ga Glu aca Tha	c ag y Arg 5 a ggg r Gly g gcc J Ala c acc r Thr 2	g tcc (g ccc (Pro / 2 aca Thr (260 tat cc Tyr (75	ccc agt Pro Ser 230 cga gar Arg Glu 45 tgc aaa Cys Lys ag atg Gln Met tg aag /al Lys	gac tgc r Asp Cy g agt ga s Ser Asp a gac acc Asp Thi 265 gat gtc c Asp Val 280	tgc cac s Cys Hi 235 c tgt ctc c Cys Le c tgc cca c cys Pro	Gln Cy: 220 aac ca is Asn (g gtc tg u Val C a cca ct o Pro L 27 gaa gge o Glu G 285 aac ta	a tgt go Gln Cys 240 c caa aa cys Gln I 255 c atg ct eu Met I 0 g aag ta	t gcg ggg tgt Ala Ala Gly Cy ag ttc caa gat Lys Phe Gln As g tac aac ccc Leu Tyr Asn Pr	720 s 768 sp 816 o 864

	305	310	315	320		
-		g Lys Cys Lys		ggg ccc tgt cg p Gly Pro Cys A 335		1008
	tgt aat ggc ata Cys Asn Gly II 340	e ggc att ggt e Gly Ile Gly (345	Glu Phe Lys	gac aca ctc tcc Asp Thr Leu S 350	ata aat er Ile Asn	1056
	gct aca aac at Ala Thr Asn Ile 355	c aaa cac ttc e Lys His Phe 360	aaa tac tgc Lys Tyr Cys 36	act gcc atc ago Thr Ala Ile Se 55	ggg gac r Gly Asp	1104
	ctt cac atc ctg Leu His Ile Leu 370	cca gtg gcc.t u Pro Val Ala I 375	tt aag ggg Phe Lys Gly 380	gat tct ttc acg Asp Ser Phe T	cgc act 1 hr Arg Thr	152
				cta aaa acc gta e Leu Lys Thr V 400		1200
-	ata aca ggc tt Ile Thr Gly Pho 40!	e Leu Leu Ile	ag gct tgg c Gln Ala Trp 110	ct gat aac tgg Pro Asp Asn Tr 415	act gac : p Thr Asp	1248.
	ctc cat gct ttc Leu His Ala Ph 420	gag aac cta g e Glu Asn Let 425	ı Glu Ile Ile	cgt ggc aga ac Arg Gly Arg Th 430	a aag caa nr Lys Gln	1296
				tg aac atc aca Leu Asn Ile Th 5		344
				ggg gat gtg at p Gly Asp Val I		1392
				a ata aac tgg aa hr Ile Asn Trp l 480		1440
		o Asn Gln Lys		atg aac aac a Met Asn Asn / 495		1488
			His Val Cy	c aat cct tta tgo s Asn Pro Leu (510		1536
				c tgt gtc tcc tg p Cys Val Ser (5		1584

gtg agc aga ggc agg gag tgc gtg gag aaa tgc aac atc ctg gag ggg

540

560

Val Ser Arg Gly Arg Glu Cys Val Glu Lys Cys Asn Ile Leu Glu Gly

gaa cca agg gag ttt gtg gaa aat tct gaa tgc atc cag tgc cat cca

Glu Pro Arg Glu Phe Val Glu Asn Ser Glu Cys Ile Gln Cys His Pro

gaa tgt ctg ccc cag gcc atg aac atc acc tgt aca ggc agg ggg cca

535

1632

1680

1728

545

Glu Cys Leu Pro Gln Ala Met Asn Ile Thr Cys Thr Gly Arg Gly Pro 565 570 575

gac aac tgc atc cag tgt gcc cac tac att gat ggc cca cac tgt gtc

Asp Asn Cys Ile Gin Cys Ala His Tyr Ile Asp Gly Pro His Cys Val

580

585

590

aag acc tgc cca gct ggc atc atg gga gag aac aac act ctg gtc tgg
Lys Thr Cys Pro Ala Gly Ile Met Gly Glu Asn Asn Thr Leu Val Trp
595 600 605

aag tat gca gat gcc aat aat gtc tgc cac cta tgc cac gcc aac tgt 1872 Lys Tyr Ala Asp Ala Asn Asn Val Cys His Leu Cys His Ala Asn Cys 610 615 620

acc tat gga tgt gct ggg cca ggt ctt caa gga tgt gaa gtg tgg cca
Thr Tyr Gly Cys Ala Gly Pro Gly Leu Gln Gly Cys Glu Val Trp Pro
625 630 635 640

tct ggg tac gtt caa tgg cag tgg atc tta aag acc ttt tgg atc taa 1968 Ser Gly Tyr Val Gln Trp Gln Trp Ile Leu Lys Thr Phe Trp Ile 645 650 655

<210> 18

<211> 655

<212> PRT

<213> 小鼠(Mus musculus)

<400> 18

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Gly Thr Ser Asn Arg Leu Thr Gln Leu Gly Thr Phe Glu Asp His Phe 35 40 45

Leu Ser Leu Gln Arg Met Tyr Asn Asn Cys Glu Val Val Leu Gly Asn 50 55 60

Leu Glu Ile Thr Tyr Val Gln Arg Asn Tyr Asp Leu Ser Phe Leu Lys
65 70 75 80

Thr Ile Gln Glu Val Ala Gly Tyr Val Leu Ile Ala Leu Asn Thr Val 85 90 95

Glu Arg Ile Pro Leu Glu Asn Leu Gln Ile Ile Arg Gly Asn Ala Leu 100 105 110

- Tyr Glu Asn Thr Tyr Ala Leu Ala Ile Leu Ser Asn Tyr Gly Thr Asn 115 120 125
- Arg Thr Gly Leu Arg Glu Leu Pro Met Arg Asn Leu Gln Glu Ile Leu 130 135 140
- Ile Gly Ala Val Arg Phe Ser Asn Asn Pro Ile Leu Cys Asn Met Asp 145 150 155 160
- Thr Ile Gin Trp Arg Asp Ile Val Gin Asn Val Phe Met Ser Asn Met 165 170 175
- Ser Met Asp Leu Gln Ser His Pro Ser Ser Cys Pro Lys Cys Asp Pro 180 185 190
- Ser Cys Pro Asn Gly Ser Cys Trp Gly Gly Gly Glu Glu Asn Cys Gln 195 200 205
- Lys Leu Thr Lys Ile Ile Cys Ala Gln Gln Cys Ser His Arg Cys Arg 210 215 220
- Gly Arg Ser Pro Ser Asp Cys Cys His Asn Gln Cys Ala Ala Gly Cys 225 230 235 240
- Thr Gly Pro Arg Glu Ser Asp Cys Leu Val Cys Gln Lys Phe Gln Asp 245 250 255
- Glu Ala Thr Cys Lys Asp Thr Cys Pro Pro Leu Met Leu Tyr Asn Pro 260 265 270
- Thr Thr Tyr Gln Met Asp Val Asn Pro Glu Gly Lys Tyr Ser Phe Gly 275 280 285
- Ala Thr Cys Val Lys Lys Cys Pro Arg Asn Tyr Val Val Thr Asp His 290 295 300
- Gly Ser Cys Val Arg Ala Cys Gly Pro Asp Tyr Tyr Glu Val Glu Glu 305 310 315 320
- Asp Gly Ile Arg Lys Cys Lys Lys Cys Asp Gly Pro Cys Arg Lys Val 325 330 335
- Cys Asn Gly Ile Gly Ile Gly Glu Phe Lys Asp Thr Leu Ser Ile Asn 340 345 350
- Ala Thr Asn Ile Lys His Phe Lys Tyr Cys Thr Ala Ile Ser Gly Asp 355 360 365

- Leu His Ile Leu Pro Val Ala Phe Lys Gly Asp Ser Phe Thr Arg Thr 370 375 380
- Pro Pro Leu Asp Pro Arg Glu Leu Glu Ile Leu Lys Thr Val Lys Glu 385 390 395 400
- Ile Thr Gly Phe Leu Leu Ile Gln Ala Trp Pro Asp Asn Trp Thr Asp 405 410 415
- Leu His Ala Phe Glu Asn Leu Glu Ile Ile Arg Gly Arg Thr Lys Gln 420 425 430
- His Gly Gln Phe Ser Leu Ala Val Val Gly Leu Asn Ile Thr Ser Leu 435 440 445
- Gly Leu Arg Ser Leu Lys Glu Ile Ser Asp Gly Asp Val Ile Ile Ser 450 455 460
- Gly Asn Arg Asn Leu Cys Tyr Ala Asn Thr Ile Asn Trp Lys Lys Leu 465 470 475 480
- Phe Gly Thr Pro Asn Gln Lys Thr Lys Ile Met Asn Asn Arg Ala Glu 485 490 495
- Lys Asp Cys Lys Ala Val Asn His Val Cys Asn Pro Leu Cys Ser Ser 500 505 510
- Glu Gly Cys Trp Gly Pro Glu Pro Arg Asp Cys Val Ser Cys Gln Asn 515 520 525
- Val Ser Arg Gly Arg Glu Cys Val Glu Lys Cys Asn Ile Leu Glu Gly 530 535 540
- Glu Pro Arg Glu Phe Val Glu Asn Ser Glu Cys Ile Gln Cys His Pro 545 550 555 560
- Glu Cys Leu Pro Gln Ala Met Asn Ile Thr Cys Thr Gly Arg Gly Pro 565 570 575
- Asp Asn Cys Ile Gln Cys Ala His Tyr Ile Asp Gly Pro His Cys Val 580 585 590
- Lys Thr Cys Pro Ala Gly Ile Met Gly Glu Asn Asn Thr Leu Val Trp 595 600 605
- Lys Tyr Ala Asp Ala Asn Asn Val Cys His Leu Cys His Ala Asn Cys

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Glu Lys Lys Val Cys Gln Gly Thr Asn Asn Lys Leu Thr Gln Leu Gly
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cac gtg gaa gac cat ttc acc agc ctg cag aga atg tac aac aac tgc
His Val Glu Asp His Phe Thr Ser Leu Gln Arg Met Tyr Asn Asn Cys
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gaa gtg gta ctg agt aac ctg gag att acc tac gtg gag cat aat cgc
Glu Val Val Leu Ser Asn Leu Glu Ile Thr Tyr Val Glu His Asn Arg
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gat ctc acc ttc ctt aag acc ata cag gag gtt gca ggc tat gtg ctc 288 Asp Leu Thr Phe Leu Lys Thr Ile Gin Glu Val Ala Gly Tyr Val Leu 85 90 95

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Ile Ala Leu Asn Met Val Asp Val Ile Pro Leu Glu Asn Leu Gln Ile

100

105

110

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ccc aaa ctg tgc aac atg gac act gtt ctc tgg aat gac atc att gat 528 Pro Lys Leu Cys Asn Met Asp Thr Val Leu Trp Asn Asp Ile Ile Asp 165 170 175
aca agc agg aag cct ctc aca gta ctt gac ttt gca agc aat ctt tct 576 Thr Ser Arg Lys Pro Leu Thr Val Leu Asp Phe Ala Ser Asn Leu Ser 180 185 190
tct tgt cca aaa tgc cat ccg aac tgc aca gaa gac cac tgc tgg ggt Ser Cys Pro Lys Cys His Pro Asn Cys Thr Glu Asp His Cys Trp Gly 195 200 205
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cca ctg gtc ctc tat aac ccc acc acc tat caa atg gat gtc aac cct 864 Pro Leu Val Leu Tyr Asn Pro Thr Thr Tyr Gln Met Asp Val Asn Pro 275 280 285
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gat ggg cta tgt agc aaa gtg tgc aat ggc att gga ata ggt gaa ctt Asp Gly Leu Cys Ser Lys Val Cys Asn Gly Ile Gly Ile Gly Glu Leu 340 345 350
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tgt acg aag atc aat ggg gat gtc agc att ctt cct gtt gca ttt cta 1152 Cys Thr Lys Ile Asn Gly Asp Val Ser Ile Leu Pro Val Ala Phe Leu

2	7	n
J	,	v

- ggg gat gcc ttc aca aag aca cta ccc ctt gac cct aag aag ctg gat 1200 Gly Asp Ala Phe Thr Lys Thr Leu Pro Leu Asp Pro Lys Lys Leu Asp 385 390 395 400
- gtc ttt aga aca gtc aaa gaa ata tca gga ttt ttg ttg att cag gcc 1248 Val Phe Arg Thr Val Lys Glu Ile Ser Gly Phe Leu Leu Ile Gln Ala 405 410 415
- tgg cct gat aat gct act gat ctc tat gct ttt gaa aat ctg gag att 1296 Trp Pro Asp Asn Ala Thr Asp Leu Tyr Ala Phe Glu Asn Leu Glu Ile 420 425 430
- atc cga ggc cga acc aag cag cac ggc cag tat tcc ctt gct gtt gtt 1344

 Ile Arg Gly Arg Thr Lys Gln His Gly Gln Tyr Ser Leu Ala Val Val

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 Asn Leu Lys Ile Gln Ser Leu Gly Leu Arg Ser Leu Lys Glu Ile Ser
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 455
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- acc atg aac tgg cgc agc ttg ttt gct act cag agt cag aaa aca aaa 1488 Thr Met Asn Trp Arg Ser Leu Phe Ala Thr Gln Ser Gln Lys Thr Lys 485 490 495
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 Ile Ile Gln Asn Arg Asn Lys Asn Asp Cys Thr Ala Asp Arg His Val
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 Cys Asp Pro Leu Cys Ser Asp Val Gly Cys Trp Gly Pro Gly Pro Phe

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- cag tgc aac atc ctg caa ggg gag cca cgt gag ttt gaa aga gac tcc
 Gln Cys Asn Ile Leu Gln Gly Glu Pro Arg Glu Phe Glu Arg Asp Ser
 545
 550
 555
 560
- aaa tgc ctc ccc tgc cac tca gag tgt ctg gta cag aac tcc act gca 1728 Lys Cys Leu Pro Cys His Ser Glu Cys Leu Val Gln Asn Ser Thr Ala 565 570 575
- tac aac aca acc tgc tct gga ccg ggc cca gac cac tgc atg aag tgt 1776 Tyr Asn Thr Thr Cys Ser Gly Pro Gly Pro Asp His Cys Met Lys Cys 580 585 590
- gcc cat ttt ata gat ggt ccc cac tgt gtg aag gcc tgc ccc gct ggg 1824 Ala His Phe Ile Asp Gly Pro His Cys Val Lys Ala Cys Pro Ala Gly 595 600 605
- gtc ctg ggt gag aat gat acc ctg gtc tgg aag tat gca gat gcc aat 1872 Val Leu Gly Glu Asn Asp Thr Leu Val Trp Lys Tyr Ala Asp Ala Asn 610 615 620
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Ala Val Cys Gln Leu Cys His Pro Asn Cys Thr Arg Gly Cys Lys Gly 625 630 635 640

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650

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His Val Glu Asp His Phe Thr Ser Leu Gln Arg Met Tyr Asn Asn Cys 50 55 60

Glu Val Val Leu Ser Asn Leu Glu Ile Thr Tyr Val Glu His Asn Arg 65 70 75 80

Asp Leu Thr Phe Leu Lys Thr Ile Gln Glu Val Ala Gly Tyr Val Leu 85 90 95

Ile Ala Leu Asn Met Val Asp Val Ile Pro Leu Glu Asn Leu Gln Ile 100 105 110

Ile Arg Gly Asn Val Leu Tyr Asp Asn Ser Phe Ala Leu Ala Val Leu , 115 120 125

Ser Asn Tyr His Met Asn Lys Thr Gln Gly Leu Arg Glu Leu Pro Met 130 135 140

Lys Arg Leu Ser Glu Ile Leu Asn Gly Gly Val Lys Ile Ser Asn Asn 145 150 155 160

Pro Lys Leu Cys Asn Met Asp Thr Val Leu Trp Asn Asp Ile Ile Asp 165 170 175

- Thr Ser Arg Lys Pro Leu Thr Val Leu Asp Phe Ala Ser Asn Leu Ser 180 185 190
- Ser Cys Pro Lys Cys His Pro Asn Cys Thr Glu Asp His Cys Trp Gly 195 200 205
- Ala Gly Glu Gln Asn Cys Gln Thr Leu Thr Lys Val Ile Cys Ala Gln 210 215 220
- Gln Cys Ser Gly Arg Cys Arg Gly Lys Val Pro Ser Asp Cys Cys His 225 230 235 240
- Asn Gln Cys Ala Ala Gly Cys Thr Gly Pro Arg Glu Ser Asp Cys Leu 245 250 255
- Ala Cys Arg Lys Phe Arg Asp Asp Ala Thr Cys Lys Asp Thr Cys Pro 260 265 270
- Pro Leu Val Leu Tyr Asn Pro Thr Thr Tyr Gln Met Asp Val Asn Pro 275 280 285
- Glu Gly Lys Tyr Ser Phe Gly Ala Thr Cys Val Arg Glu Cys Pro His 290 295 300
- Asn Tyr Val Val Thr Asp His Gly Ser Cys Val Arg Ser Cys Asn Thr 305 310 315 320
- Asp Thr Tyr Glu Val Glu Glu Asn Gly Val Arg Lys Cys Lys Lys Cys 325 330 335
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- Gly Asp Ala Phe Thr Lys Thr Leu Pro Leu Asp Pro Lys Lys Leu Asp 385 390 395 400
- Val Phe Arg Thr Val Lys Glu Ile Ser Gly Phe Leu Leu Ile Gln Ala 405 410 415
- Trp Pro Asp Asn Ala Thr Asp Leu Tyr Ala Phe Glu Asn Leu Glu Ile 420 425 430

- Ile Arg Gly Arg Thr Lys Gln His Gly Gln Tyr Ser Leu Ala Val 435 440 445
- Asn Leu Lys Ile Gln Ser Leu Gly Leu Arg Ser Leu Lys Glu Ile Ser 450 455 460
- Asp Gly Asp Ile Ala Ile Met Lys Asn Lys Asn Leu Cys Tyr Ala Asp 465 470 475 480
- Thr Met Asn Trp Arg Ser Leu Phe Ala Thr Gin Ser Gin Lys Thr Lys 485 490 495
- Ile Ile Gln Asn Arg Asn Lys Asn Asp Cys Thr Ala Asp Arg His Val $500 \hspace{1.5cm} 505 \hspace{1.5cm} 510$
- Cys Asp Pro Leu Cys Ser Asp Val Gly Cys Trp Gly Pro Gly Pro Phe 515 520 525
- His Cys Phe Ser Cys Arg Phe Phe Ser Arg Gln Lys Glu Cys Val Lys 530 535 540
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- Tyr Asn Thr Thr Cys Ser Gly Pro Gly Pro Asp His Cys Met Lys Cys 580 585 590
- Ala His Phe Ile Asp Gly Pro His Cys Val Lys Ala Cys Pro Ala Gly 595 600 605
- Val Leu Gly Glu Asn Asp Thr Leu Val Trp Lys Tyr Ala Asp Ala Asn 610 615 620
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atc acc aca agc tca tcg gtc agc aat gcc ggc tat gtg gat aat ggc 1 Ile Thr Thr Ser Ser Ser Val Ser Asn Ala Gly Tyr Val Asp Asn Gly 35 40 45	44
aat atg aaa gtc tgc atc ggc act aaa tct cgg ctc tcc gtg ccc tcc 19 Asn Met Lys Val Cys Ile Gly Thr Lys Ser Arg Leu Ser Val Pro Ser 50 55 60	2
aac aag gaa cat cat tac cga aac ctc aga gat cgg tac acg aac tgt Asn Lys Glu His His Tyr Arg Asn Leu Arg Asp Arg Tyr Thr Asn Cys 65 70 75 80	240
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ttg gac ctc agc ttc cta gac aac ata cgg gag gtc acc ggc tat att Leu Asp Leu Ser Phe Leu Asp Asn Ile Arg Glu Val Thr Gly Tyr Ile 100 105 110	36
ctg atc agt cat gtg gac gtt aag aaa gtg gtg ttt ccc aaa cta caa 38 Leu Ile Ser His Val Asp Val Lys Lys Val Val Phe Pro Lys Leu Gin 115 120 125	34
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4	_	J

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	agc ctg Ser Lei 465	gag u Glu	Met	cgc aal Arg As 70	t ctg a sn Leu	ag cag Lys G 475	att a In Ile	agt to Ser S	c ggc a er Gly 480	gt gtg g Ser Val	tc Val	1440
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	tcc aat Ser Asr	n Ala	ac aa Tyr I 65	g ttt g ys Phe	ac aal Asp / 570	\sn Ar	acg to g Thr	c aag Cys L 575	ata tg ys Ile	c cat co Cys His	a Pro	1728
	gag tgc Glu Cys	cgg Arg 580	act t Thr (gc aat Cys As	gga g n Gly A 585	ct gga Ala Gly	/ Ala /	gat ca Asp H 90	c tgc c is Cys	ag gag t Gin Giu	tgc Cys	1776
	Val His	gtg a Val A 95	gg g .rg A	ac ggt sp Gly 60	Gln Hi	ac tgt is Cys	gtg to Val S 605	er Glu	tgc co L Cys P	cg aag a Pro Lys A	ac \sn	1824
	aag tac Lys Tyr 610	aac (Asn	Asp /	gt ggt Arg Gly 615	gtc tg / Val C	ys Arg	gag t g Glu 20	gc cad Cys H	gcc a lis Ala	cc tgc ga Fhr Cys	at Asp	1872
	gga tgc Gly Cys 625	act o	Gly F	cc aag ro Lys 30	gac a Asp T	cc atc hr Ile 635	ggc a Gly Il	e Gly	a gcg t Ala Cy 640	gt acg a s Thr Th	icg ir	1920
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	ctg aag Leu Lys	gac g Asp 6	gac a Asp I	Lys Cy	ccc ga s Pro <i>f</i> 665	at ggg \sp Gl	y Tyr	tc tgg Phe T 70	gag ta rp Glu	at gtg ca Tyr Val	t His	2016
	ccg caa Pro Gln 67	Glu C	cag g Sin G	ga tcg ly Ser 68	Leu Ly	ag cca /s Pro	ttg g Leu / 685	jcc gg Na Gly	c aga g y Arg A	gca gtt t Na Val C	gc ys	2064
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Arg Lys Cys His Pro Leu Cys Glu Leu Cys Thr Asn Tyr Gly Tyr His 690 695 700	
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865 870 875 880	
865 870 875 880 ttg cgc att gtc aag gac gcc gag ttg cgc aag ggc gga gtc ctt gga Leu Arg Ile Val Lys Asp Ala Glu Leu Arg Lys Gly Gly Val Leu Gly	
865 870 875 880 ttg cgc att gtc aag gac gcc gag ttg cgc aag ggc gga gtc ctt gga Leu Arg Ile Val Lys Asp Ala Glu Leu Arg Lys Gly Gly Val Leu Gly 885 890 895 atg gga gcc ttt gga cga gtg tac aag ggc gtt tgg gtg ccg gag ggt Met Gly Ala Phe Gly Arg Val Tyr Lys Gly Val Trp Val Pro Glu Gly	

Ser Glu Glu His Val Asn Leu Leu L 945 950 95	ys Leu Leu Ala Val Cys Met Ser	2880
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Asn Met Lys Val Cys Ile Gly Thr Lys Ser Arg Leu Ser Val Pro Ser 50 55 60

Asn Lys Glu His His Tyr Arg Asn Leu Arg Asp Arg Tyr Thr Asn Cys 65 70 75 80

Thr Tyr Val Asp Gly Asn Leu Lys Leu Thr Trp Leu Pro Asn Glu Asn 85 90 95

Leu Asp Leu Ser Phé Leu Asp Asn Ile Arg Glu Val Thr Gly Tyr Ile

105

- Leu Ile Ser His Val Asp Val Lys Lys Val Val Phe Pro Lys Leu Gln
 115 120 125
- Ile Ile Arg Gly Arg Thr Leu Phe Ser Leu Ser Val Glu Glu Glu Lys 130 135 140
- Tyr Ala Leu Phe Vai Thr Tyr Ser Lys Met Tyr Thr Leu Glu Ile Pro 145 150 155 160
- Asp Leu Arg Asp Val Leu Asn Gly Gln Val Gly Phe His Asn Asn Tyr 165 170 175
- Asn Leu Cys His Met Arg Thr Ile Gln Trp Ser Glu Ile Val Ser Asn 180 185 190
- Gly Thr Asp Ala Tyr Tyr Asn Tyr Asp Phe Thr Ala Pro Glu Arg Glu 195 200 205
- Cys Pro Lys Cys His Glu Ser Cys Thr His Gly Cys Trp Gly Glu Gly 210 215 220
- Pro Lys Asn Cys Gln Lys Phe Ser Lys Leu Thr Cys Ser Pro Gln Cys 225 230 235 240
- Ala Gly Gly Arg Cys Tyr Gly Pro Lys Pro Arg Glu Cys Cys His Leu 245 250 255
- Phe Cys Ala Gly Gly Cys Thr Gly Pro Thr Gln Lys Asp Cys Ile Ala 260 265 270
- Cys Lys Asn Phe Phe Asp Glu Ala Val Ser Lys Glu Glu Cys Pro Pro 275 280 285
- Met Arg Lys Tyr Asn Pro Thr Thr Tyr Val Leu Glu Thr Asn Pro Glu 290 295 300
- Gly Lys Tyr Ala Tyr Gly Ala Thr Cys Val Lys Glu Cys Pro Gly His 305 310 315 320
- Leu Leu Arg Asp Asn Gly Ala Cys Val Arg Ser Cys Pro Gln Asp Lys 325 330 335
- Met Asp Lys Gly Gly Glu Cys Val Pro Cys Asn Gly Pro Cys Pro Lys 340 345 350

- Thr Cys Pro Gly Val Thr Val Leu His Ala Gly Asn Ile Asp Ser Phe 355 360 365
- Arg Asn Cys Thr Val Ile Asp Gly Asn Ile Arg Ile Leu Asp Gln Thr 370 375 380
- Phe Ser Gly Phe Gln Asp Val Tyr Ala Asn Tyr Thr Met Gly Pro Arg 385 390 395 400
- Tyr Ile Pro Leu Asp Pro Glu Arg Arg Glu Val Phe Ser Thr Val Lys 405 410 415
- Glu Ile Thr Gly Tyr Leu Asn Ile Glu Gly Thr His Pro Gln Phe Arg 420 425 430
- Asn Leu Ser Tyr Phe Arg Asn Leu Glu Thr Ile His Gly Arg Gln Leu 435 440 445
- Met Glu Ser Met Phe Ala Ala Leu Ala Ile Val Lys Ser Ser Leu Tyr 450 455 460
- Ser Leu Glu Met Arg Asn Leu Lys Gln Ile Ser Ser Gly Ser Val Val 465 470 475 480
- Ile Gln His Asn Arg Asp Leu Cys Tyr Val Ser Asn Ile Arg Trp Pro 485 490 495
- Ala Ile Gln Lys Glu Pro Glu Gln Lys Val Trp Val Asn Glu Asn Leu 500 505 510
- Arg Ala Asp Leu Cys Glu Lys Asn Gly Thr Ile Cys Ser Asp Gln Cys 515 520 525
- Asn Glu Asp Gly Cys Trp Gly Ala Gly Thr Asp Gln Cys Leu Thr Cys 530 535 540
- Lys Asn Phe Asn Phe Asn Gly Thr Cys Ile Ala Asp Cys Gly Tyr Ile 545 550 555 560
- Ser Asn Ala Tyr Lys Phe Asp Asn Arg Thr Cys Lys Ile Cys His Pro 565 570 575
- Glu Cys Arg Thr Cys Asn Gly Ala Gly Ala Asp His Cys Gln Glu Cys 580 585 590
- Val His Val Arg Asp Gly Gln His Cys Val Ser Glu Cys Pro Lys Asn 595 600 605

- Lys Tyr Asn Asp Arg Gly Val Cys Arg Glu Cys His Ala Thr Cys Asp 610 615 620
- Gly Cys Thr Gly Pro Lys Asp Thr Ile Gly Ile Gly Ala Cys Thr Thr 625 630 635 640
- Cys Asn Leu Ala Ile Ile Asn Asn Asp Ala Thr Val Lys Arg Cys Leu 645 650 655
- Leu Lys Asp Asp Lys Cys Pro Asp Gly Tyr Phe Trp Glu Tyr Val His 660 665 670
- Pro Gln Glu Gln Gly Ser Leu Lys Pro Leu Ala Gly Arg Ala Val Cys 675 680 685
- Arg Lys Cys His Pro Leu Cys Glu Leu Cys Thr Asn Tyr Gly Tyr His 690 695 700
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- Glu Thr Glu Cys Pro Ala Asp His Tyr Thr Asp Glu Glu Gln Arg Glu 725 730 735
- Cys Phe Gln Arg His Pro Glu Cys Asn Gly Cys Thr Gly Pro Gly Ala 740 745 750
- Asp Asp Cys Lys Ser Cys Arg Asn Phe Lys Leu Phe Asp Ala Asn Glu 755 760 765
- Thr Gly Pro Tyr Val Asn Ser Thr Met Phe Asn Cys Thr Ser Lys Cys 770 775 780
- Pro Leu Glu Met Arg His Val Asn Tyr Gln Tyr Thr Ala Ile Gly Pro 785 790 795 800
- Tyr Cys Ala Ala Ser Pro Pro Arg Ser Ser Lys Ile Thr Ala Asn Leu 805 810 815
- Asp Val Asn Met Ile Phe Ile Ile Thr Gly Ala Val Leu Val Pro Thr 820 825 830
- Ile Cys Ile Leu Cys Val Val Thr Tyr Ile Cys Arg Gln Lys Gln Lys 835 840 845
- Ala Lys Lys Glu Thr Val Lys Met Thr Met Ala Leu Ser Gly Cys Glu 850 855 860

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- Glu Asn Val Lys Ile Pro Val Ala Ile Lys Glu Leu Leu Lys Ser Thr 915 920 925
- Gly Ala Glu Ser Ser Glu Glu Phe Leu Arg Glu Ala Tyr Ile Met Ala 930 935 940
- Ser Glu Glu His Val Asn Leu Leu Lys Leu Leu Ala Val Cys Met Ser 945 950 955 960
- Ser Gln Met Met Leu Ile Thr Gln Leu Met Pro Leu Gly Cys Leu Leu 965 970 975
- Asp Tyr Val Arg Asn Asn Arg Asp Lys Ile Gly Ser Lys Ala Leu Leu 980 985 990
- Asn Trp Ser Thr Gln Ile Ala Lys Gly Met Ser Tyr Leu Glu Glu Lys 995 1000 1005
- Arg Leu Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Gln 1010 1015 1020
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- Pro Ile Lys Trp Leu Ala Leu Glu Cys Ile Arg Asn Arg Val Phe 1055 1060 1065
- Thr Ser Lys Ser Asp Val Trp Ala Phe Gly Val Thr Ile Trp Glu 1070 1075 1080
- Leu Leu Thr Phe Gly Gln Arg Pro His Glu Asn Ile Pro Ala Lys 1085 1090 1095
- Asp Ile Pro Asp Leu Ile Glu Val Gly Leu Lys Leu Glu Gln Pro

1105

1110

Glu Ile Cys Ser Leu Asp Ile Tyr Cys Thr Leu Leu Ser Cys Trp 1115 1120 1125

His Leu Asp Ala Ala Met Arg Pro Thr Phe Lys Gln Leu Thr Thr 1130 1135 1140

Val Phe Ala Glu Phe Ala Arg. Asp Pro Gly Arg Tyr Leu Ala Ile 1145 1150 1155

Pro Gly Asp Lys Phe Thr Arg Leu Pro Ala Tyr Thr Ser Gln Asp 1160 1165 1170

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tcc aac aag gaa cat cat tac cga aac ctc aga gat cgg tac acg aac 384 Ser Asn Lys Glu His His Tyr Arg Asn Leu Arg Asp Arg Tyr Thr Asn 115 120 125
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Cys Thr Tyr Val Asp Gly Asn Leu Lys Leu Thr Trp Leu Pro Asn Glu

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Arg	tac Tyr 50	ata Ile	ccg Pro I	Leu .	gat c Asp f 55	cc ga Pro G	ig co lu A	ga c rg A 460	Arg (jag (Slu \	gtg † Val F	ttc to the S	c acg Ser Ti	g gtg hr Val		1392
aag Lys 465	Glu	ato Ile	Thr (ggg Gly 470	tat (Γyr L	ctg a eu As	at a sn II 47	e Gl	ag g lu Gl	iga a iy Ti	acc (nr Hi 48	is Pro	cg ca o Gln	g ttc Phe		1440
cgg Arg	aat Asn	Leu	tcg Ser 85	tac i Tyr	tt cg Phe	c aat Arg / 490	Asn	gaa Leu	a aca Glu	a att Thr 49	· Ile	ggc His (cgc Sly A	cag rg Glr	1	488
ctg a	atg Met	gag Glu 500	Ser	atg Mei	t Phe	cc gc Ala / 605	t ttg Ala I	gc Leu	Ala	c gti Ile \ 10	t aag /al L	g tca ys S	tcc d er Se	tg er Leu	15	536
tac a Tyr :	agc Ser 51	Leu	gag Glu	atg Met	cgc a Arg 520	at ct Asn I	g aa .eu	Lys	ag a Gln 525	tt a	gt to Ser :	c gg Ser (c agt Sly S	: gtg er Val	:	1584
Val I	atc d le G 30	ag Sin F	cat a dis A	sn A	ga g Arg A: 35	ac ct sp Le	c tge u C	c ta ys T 540	yr V	a ag 'al S	c aa Ser A	t atc sn II	cgt e Arg	tgg 3 Trp	1	632
ccg (Pro 7 545	gcc Ala I	att d lie C	in L	aag ys G 550	gag o Slu Pr	ccc ga	aa c I Gli 55	n Ly	aag g s Va	gtg i	tgg p Va 56	ıl Asr	ac ga Glu	ag aat Asn	Ė	1680
ctc a Leu <i>i</i>	igg i Arg	Ala	gat Asp 65	cta 1 Leu	tgc ga Cys	ag aa Glu L 570	.ys /	at g Asn	ga a Gly	cc a Thr 575	Ile (gc to Cys S	g gat Ser A	cag sp Glr	1	1728
tgc a Cys <i>i</i>	٩sn	gag Glu 580	Asp	ggc Gly	Cys	gg gg Trp (85	ga g Sly A	ct g Ala (gc a Gly 7 59	Thr /	gat (Asp	cag t Gln (gc ct Cys L	t acc eu Th	r	1776
tgc a Cys I	ag : _ys : 59!	Asn	Phe	Asn	Phe	t ggc Asn	Gly '	Thr	Cvs	Ile.	gad Ala	tgt Asp (ggt t Cys C	at Sly Ty	18 r	24
ata t Ile S 61	er A	at g sn A	icc ta Ala T	ac a yr L 61	ys Pr	t gac ne As	p As	aga sn A 620	rg T	tgo hr C	c aag Cys I	g ata .ys Il	tgc e Cy	cat s His	18	872
cca g Pro G 525	iag i Slu (tgc (Cys :	Arg	act t Thr 30	gc aa Cys <i>A</i>	at gg Asn G	a go ily A 63!	la G	ga go Sly A	ca g la A	at c sp l 640	lis C	c cag ys Gl	gag In Glu		1920
tgc g Cys \	tc c /al l	tis \	tg ag /al A 45	gg g irg A	ac gg sp G	gt cag lly Gl 650	n Hi	c tg s Cy	t gtg /s Va	g tco al Se 655	er G	g tgc lu Cy	ccg s Pro	aag Lys	1	
aca Asn L	.ys	tac a Fyr <i>i</i> 560	aac g Asn <i>i</i>	gat o Asp	Arg (gt gto Gly V 55	tgo al C	c cg ys <i>A</i>	a ga Arg (67	Slu (c ca Cys	ıc gcı His A	c acc lla Th	tgc or Cys	2	2016
gat g Asp C	ga t Sly (gc a Cys	ect g Thr (gg d Gly I	cc aa Pro L	ag ga ys As	ic ac ip TI	c at	tc gg le Gl	oc at	tt gg e Gly	ja gc / Ala	g tgt Cys	acg Thr		2064

			•				
6	575	68	30	685			
acg tg Thr Cy 690	's Asn L	g gcc att a eu Ala Ile 695	Ile Asn As	gac gcc ao n Asp Ala ^{^-} 700	ca gta aaa cgc Thr Val Lys Arg	tgc 3 Cys	2112
ctg ctg Leu Le 705	aag ga u Lys A	ac gac aag sp Asp Ly 710	tgc ccc ga s Cys Pro A 71	Asp Gly Tyı	tc tgg gag tat r Phe Trp Glu T 720	gtg 「yr Val	2160
cat cco	caa ga Gln Gl 72	u Gin Giy	a tog otg aa Ser Leu Ly 730	g cca ttg s Pro Leu 73	gcc ggc aga gc Ala Gly Arg Ala 5	a gtt Val	2208
tgc cga Cys Ar	a aag to g Lys C 740	gc cat ccc ys His Pro	ctt tgc gag Leu Cys G 745	ctg tgc ac lu Leu Cys 750	g aac tac gga Thr Asn Tyr G	tac Sly Tyr	2256
HIS GIL	a cag gl ı Gln Va 55	g tgc tcc al Cys Ser 76	Lys Cys Th	cac tac a r His Tyr L 765	ag cga cgg gag .ys Arg Arg Glu	g cag u Gln	2304
tgc gag Cys Glo 770	g acc ga u Thr G	ag tgt ccg lu Cys Pro 775	Ala Asp Hi	tac acg g s Tyr Thr / 780	at gag gag cag Asp Glu Glu Gli	g cgc n Arg	2352
gag tgo Glu Cys 785	c ttc cag s Phe G	g cgc cac In Arg His 790	ccg gaa tgc Pro Glu Cy 795	's Asn Gly	gc acg gigt ccg Cys Thr Gly Pr 800	ggt o Gly	2400
gcc gad Ala Asp	gat tg Asp Cy 805	ys Lys Ser	gc cgc aac Cys Arg A 810	ttt aag ttg sn Phe Lys 81!	g ttc gac gcg a Leu Phe Asp A S	at 2 Ala Asn	448
gag acq Glu Thr	ggt co Gly Pro 820	o Tyr Val /	aac tcc acg Asn Ser Thi 825	atg ttc aa Met Phe / 830	t tgc acc tcg a Asn Cys Thr Se	ag 2 er Lys	2496
Cys Pro	ttg gag Leu Gl 35	atg cga c u Met Arg 840	His Val As	tat cag tad n Tyr Gln ⁻ 845	acg gcc att g Tyr Thr Ala Ile	ga 2 Gly	2544
ccc tac Pro Tyr 850	tgc gca Cys Ala	gct agt c Ala Ser I 855	Pro Pro Arg	agc agc a Ser Ser L 60	ag ata act gcc ys Ile Thr Ala /	aat Asn	2592
ctg gat Leu Asp 865	gtg aad Val As	atg atc t n Met Ile 870	tc att atc a Phe Ile Ile 875	Thr Gly Ala	gtt ctg gtg ccg a Val Leu Val P	264 ro	40

acg atc tgc atc ctc tgc gtg gtc aca tac att tgt cgg caa aag caa

Thr Ile Cys Ile Leu Cys Val Val Thr Tyr Ile Cys Arg Gln Lys Gln

aag gcc aag aag gaa aca gtc aag atg acc atg gct ctg tct ggc tgc Lys Ala Lys Lys Glu Thr Val Lys Met Thr Met Ala Leu Ser Gly Cys

gag gat tcc gag ccg ctg cgt ccc tcg aac att gga gcc aac cta tgc Glu Asp Ser Glu Pro Leu Arg Pro Ser Asn Ile Gly Ala Asn Leu Cys

890

905

2688

2736

885

930 935 940
gga atg gga gcc ttt gga cga gtg tac aag ggc gtt tgg gtg ccg gag 2880 Gly Met Gly Ala Phe Gly Arg Val Tyr Lys Gly Val Trp Val Pro Glu 945 950 955 960
ggt gag aac gtc aag att cca gtg gcc att aag gag ctg ctc aag tcc 2928 Gly Glu Asn Val Lys Ile Pro Val Ala Ile Lys Glu Leu Leu Lys Ser 965 970 975
aca ggc gcc gag tca agc gaa gag ttc ctc cgc gaa gcc tac atc atg Thr Gly Ala Glu Ser Ser Glu Glu Phe Leu Arg Glu Ala Tyr Ile Met 980 985 990
gcc tct gag gag cac gtt aat ctg ctg aag ctc ctg gcc gtg tgc atg 3024 Ala Ser Glu Glu His Val Asn Leu Leu Lys Leu Leu Ala Val Cys Met 995 1000 1005
tcc tca caa atg atg cta atc acg caa ctg atg ccg ctt ggc tgc 3069 Ser Ser Gln Met Met Leu Ile Thr Gln Leu Met Pro Leu Gly Cys 1010 1015 1020
ctg ttg gac tat gtg cga aat aac cgg gac aag atc ggc tct aag 3114 Leu Leu Asp Tyr Val Arg Asn Asn Arg Asp Lys Ile Gly Ser Lys 1025 1030 1035
gct ctg ctc aac tgg agc acg caa atc gcc aag ggc atg tcg tat 3159 Ala Leu Leu Asn Trp Ser Thr Gln Ile Ala Lys Gly Met Ser Tyr 1040 1045 1050
ctg gag gag aag cga ctg gtc cac aga gac ttg gct gcc cgc aat Leu Glu Glu Lys Arg Leu Val His Arg Asp Leu Ala Ala Arg Asn 1055 1060 1065
gtc ctg gtg cag act ccc tcg ctg gtg aag atc acc gac ttt ggg 3249 Val Leu Val Gln Thr Pro Ser Leu Val Lys Ile Thr Asp Phe Gly 1070 1075 1080
ctg gcc aag ttg ctg agc agc gat tcc aat gag tac aag gct gct 3294 Leu Ala Lys Leu Ser Ser Asp Ser Asn Glu Tyr Lys Ala Ala 1085 1090 1095
ggc ggc aag atg ccc atc aag tgg ttg gca ctg gag tgc atc cgc 3339 Gly Gly Lys Met Pro Ile Lys Trp Leu Ala Leu Glu Cys Ile Arg 1100 1105 1110
aat cgt gta ttc acc agc aag tcc gat gtc tgg gcc ttt ggt gtg 3384 Asn Arg Val Phe Thr Ser Lys Ser Asp Val Trp Ala Phe Gly Val 1115 1120 1125
acc att tgg gaa ctg ctg acc ttt ggc cag cgt cca cac gag aac 3429 Thr Ile Trp Glu Leu Leu Thr Phe Gly Gln Arg Pro His Glu Asn 1130 1135 1140
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ctg gag cag ccg gag att tgt tcg ctg gac att tac tgt aca ctg 3519 Leu Glu Gln Pro Glu Ile Cys Ser Leu Asp Ile Tyr Cys Thr Leu 1160 1165 1170

ctc tcg tgc tgg cac ttg gat gcc gcc atg cgt cca acc ttc aag 3564 Leu Ser Cys Trp His Leu Asp Ala Ala Met Arg Pro Thr Phe Lys 1175 1180 1185

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Tyr Leu Ala Ile Pro Gly
1205

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<210> 25

<211> 1209

<212> PRT

<213> 大鼠(Rattus norvegicus)

<400> 25

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Leu Leu Leu Ala His Cys Ile Cys Ile Trp Pro Ala Ser Ala Ala Arg 20 25 30

Asp Arg Tyr Ala Arg Gln Asn Asn Arg Gln Arg His Gln Asp Ile Asp 35 40 45

Arg Asp Arg Asp Arg Phe Leu Tyr Arg Ser Ser Ser Ala Gln 50 55 60

- Asn Arg Gin Arg Gly Gly Ala Asn Phe Ala Leu Gly Leu Gly Ala Asn 65 70 75 80
- Gly Val Thr Ile Pro Thr Ser Leu Glu Asp Lys Asn Lys Asn Glu Phe 85 90 95
- Val Lys Gly Lys Ile Cys Ile Gly Thr Lys Ser Arg Leu Ser Val Pro 100 105 110
- Ser Asn Lys Glu His His Tyr Arg Asn Leu Arg Asp Arg Tyr Thr Asn 115 120 125
- Cys Thr Tyr Val Asp Gly Asn Leu Lys Leu Thr Trp Leu Pro Asn Glu 130 135 140
- Asn Leu Asp Leu Ser Phe Leu Asp Asn Ile Arg Glu Val Thr Gly Tyr 145 150 155 160
- Ile Leu Ile Ser His Val Asp Val Lys Lys Val Val Phe Pro Lys Leu 165 170 175
- Gln Ile Ile Arg Gly Arg Thr Leu Phe Ser Leu Ser Val Glu Glu Glu 180 185 190
- Lys Tyr Ala Leu Phe Val Thr Tyr Ser Lys Met Tyr Thr Leu Glu Ile 195 200 205
- Pro Asp Leu Arg Asp Val Leu Asn Gly Gln Val Gly Phe His Asn Asn 210 215 220
- Tyr Asn Leu Cys His Met Arg Thr Ile Gln Trp Ser Glu Ile Val Ser 225 230 235 240
- Asn Gly Thr Asp Ala Tyr Tyr Asn Tyr Asp Phe Thr Ala Pro Glu Arg 245 250 255
- Glu Cys Pro Lys Cys His Glu Ser Cys Thr His Gly Cys Trp Gly Glu 260 265 270
- Gly Pro Lys Asn Cys Gln Lys Phe Ser Lys Leu Thr Cys Ser Pro Gln 275 280 285
- Cys Ala Gly Gly Arg Cys Tyr Gly Pro Lys Pro Arg Glu Cys Cys His 290 295 300
- Leu Phe Cys Ala Gly Gly Cys Thr Gly Pro Thr Gln Lys Asp Cys Ile 305 310 315 320

- Ala Cys Lys Asn Phe Phe Asp Glu Ala Val Ser Lys Glu Glu Cys Pro 325 330 335
- Pro Met Arg Lys Tyr Asn Pro Thr Thr Tyr Val Leu Glu Thr Asn Pro 340 345 350
- Glu Gly Lys Tyr Ala Tyr Gly Ala Thr Cys Val Lys Glu Cys Pro Gly 355 360 365
- His Leu Leu Arg Asp Asn Gly Ala Cys Val Arg Ser Cys Pro Gln Asp 370 375 380
- Lys Met Asp Lys Gly Gly Glu Cys Val Pro Cys Asn Gly Pro Cys Pro 385 390 395 400
- Lys Thr Cys Pro Gly Val Thr Val Leu His Ala Gly Asn Ile Asp Ser 405 410 415
- Phe Arg Asn Cys Thr Val Ile Asp Gly Asn Ile Arg Ile Leu Asp Gln 420 425 430
- Thr Phe Ser Gly Phe Gln Asp Val Tyr Ala Asn Tyr Thr Met Gly Pro 435 440 445
- Arg Tyr Ile Pro Leu Asp Pro Glu Arg Arg Glu Val Phe Ser Thr Val 450 455 460
- Lys Glu Ile Thr Gly Tyr Leu Asn Ile Glu Gly Thr His Pro Gln Phe 465 470 475 480
- Arg Asn Leu Ser Tyr Phe Arg Asn Leu Glu Thr Ile His Gly Arg Gln 485 490 495
- Leu Met Glu Ser Met Phe Ala Ala Leu Ala Ile Val Lys Ser Ser Leu 500 505 510
- Tyr Ser Leu Glu Met Arg Asn Leu Lys Gln Ile Ser Ser Gly Ser Val 515 520 525
- Val Ile Gln His Asn Arg Asp Leu Cys Tyr Val Ser Asn Ile Arg Trp 530 535 540
- Pro Ala Ile Gln Lys Glu Pro Glu Gln Lys Val Trp Val Asn Glu Asn 545 550 555 560
- Leu Arg Ala Asp Leu Cys Glu Lys Asn Gly Thr Ile Cys Ser Asp Gln 565 570 575

- Cys Asn Glu Asp Gly Cys Trp Gly Ala Gly Thr Asp Gln Cys Leu Thr 580 585 590
- Cys Lys Asn Phe Asn Phe Asn Gly Thr Cys Ile Ala Asp Cys Gly Tyr 595 600 605
- Ile Ser Asn Ala Tyr Lys Phe Asp Asn Arg Thr Cys Lys Ile Cys His 610 615 620
- Pro Glu Cys Arg Thr Cys Asn Gly Ala Gly Ala Asp His Cys Gln Glu 625 630 635 640
- Cys Val His Val Arg Asp Gly Gln His Cys Val Ser Glu Cys Pro Lys 645 650 655
- Asn Lys Tyr Asn Asp Arg Gly Val Cys Arg Glu Cys His Ala Thr Cys 660 665 670
- Asp Gly Cys Thr Gly Pro Lys Asp Thr Ile Gly Ile Gly Ala Cys Thr 675 680 685
- Thr Cys Asn Leu Ala Ile Ile Asn Asn Asp Ala Thr Val Lys Arg Cys 690 695 700
- Leu Leu Lys Asp Asp Lys Cys Pro Asp Gly Tyr Phe Trp Glu Tyr Val 705 710 715 720
- His Pro Gln Glu Gln Gly Ser Leu Lys Pro Leu Ala Gly Arg Ala Val 725 730 735
- Cys Arg Lys Cys His Pro Leu Cys Glu Leu Cys Thr Asn Tyr Gly Tyr 740 745 750
- His Glu Gln Val Cys Ser Lys Cys Thr His Tyr Lys Arg Arg Glu Gln 755 760 765
- Cys Glu Thr Glu Cys Pro Ala Asp His Tyr Thr Asp Glu Glu Gln Arg 770 775 780
- Glu Cys Phe Gln Arg His Pro Glu Cys Asn Gly Cys Thr Gly Pro Gly 785 790 795 800
- Ala Asp Asp Cys Lys Ser Cys Arg Asn Phe Lys Leu Phe Asp Ala Asn 805 810 815
- Glu Thr Gly Pro Tyr Val Asn Ser Thr Met Phe Asn Cys Thr Ser Lys 90

825

- Cys Pro Leu Glu Met Arg His Val Asn Tyr Gln Tyr Thr Ala Ile Gly 835 840 845
- Pro Tyr Cys Ala Ala Ser Pro Pro Arg Ser Ser Lys Ile Thr Ala Asn 850 855 860
- Leu Asp Val Asn Met Ile Phe Ile Ile Thr Gly Ala Val Leu Val Pro 865 870 875 880
- Thr Ile Cys Ile Leu Cys Val Val Thr Tyr Ile Cys Arg Gln Lys Gln 885 890 895
- Lys Ala Lys Lys Glu Thr Val Lys Met Thr Met Ala Leu Ser Gly Cys 900 905 910
- Glu Asp Ser Glu Pro Leu Arg Pro Ser Asn Ile Gly Ala Asn Leu Cys 915 920 925
- Lys Leu Arg Ile Val Lys Asp Ala Glu Leu Arg Lys Gly Gly Val Leu 930 935 940
- Gly Met Gly Ala Phe Gly Arg Val Tyr Lys Gly Val Trp Val Pro Glu 945 950 955 960
- Gly Glu Asn Val Lys Ile Pro Val Ala Ile Lys Glu Leu Leu Lys Ser 965 970 975
- Thr Gly Ala Glu Ser Ser Glu Glu Phe Leu Arg Glu Ala Tyr Ile Met 980 985 990
- Ala Ser Glu Glu His Val Asn Leu Leu Lys Leu Leu Ala Val Cys Met 995 1000 1005
- Ser Ser Gln Met Met Leu Ile Thr Gln Leu Met Pro Leu Gly Cys 1010 1015 1020
- Leu Leu Asp Tyr Val Arg Asn Asn Arg Asp Lys Ile Gly Ser Lys 1025 1030 1035
- Ala Leu Leu Asn Trp Ser Thr Gln Ile Ala Lys Gly Met Ser Tyr 1040 1045 1050
- Leu Glu Lys Arg Leu Val His Arg Asp Leu Ala Ala Arg Asn 1055 1060 1065

Val Leu Val Gln Thr Pro Ser Leu Val Lys Ile Thr Asp Phe Gly 1070 1075 1080

Leu Ala Lys Leu Leu Ser Ser Asp Ser Asn Glu Tyr Lys Ala Ala 1085 1090 1095

Gly Gly Lys Met Pro Ile Lys Trp Leu Ala Leu Glu Cys Ile Arg 1100 1105 1110

Asn Arg Val Phe Thr Ser Lys Ser Asp Val Trp Ala Phe Gly Val 1115 1120 1125

Thr Ile Trp Glu Leu Leu Thr Phe Gly Gln Arg Pro His Glu Asn 1130 1135 1140

Ile Pro Ala Lys Asp Ile Pro Asp Leu Ile Glu Val Gly Leu Lys 1145 1150 1155

Leu Glu Gln Pro Glu Ile Cys Ser Leu Asp Ile Tyr Cys Thr Leu 1160 1165 1170

Leu Ser Cys Trp His Leu Asp Ala Ala Met Arg Pro Thr Phe Lys 1175 1180 1185

Gin Leu Thr Thr Val Phe Ala Glu Phe Ala Arg Asp Pro Gly Arg 1190 1195 1200

Tyr Leu Ala Ile Pro Gly 1205

<210> 26

<211> 3576

<212> DNA

<213> 斑马鱼(Danio rerio)

<220>

<221> CDS

<222> (1)..(3573)

<223>

<400> 26

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ggg Gly	g ago Ser	ttc Phe 20	tgc g : Cys .	cg ad Ala T	cg ccg hr Pro 25	gaa a Glu L	ys Lys	a gtg s Val (80	tgt cag Cys Gln	gga g Gly Al	ca aac a Asn	96	
aac Asr	aaa Lys 35	Leu	act c	Leu l	g gga a Leu Gly 0	acg gtg / Thr V	g gaa /al Gl 45	gac c u Asp	at tat o His Tyı	cag gtt Gln Va	ctg al Leu	144	
Leu	aga ı Arg 50	atg Met	tac a	ga aa Arg <i>A</i> 55	ac tgc Asn Cy	act gtg s Thr \ 6	Jal Va	ctg ga al Leu	g aac d Glu As	tg gaa n Leu C	att Glu Ile	192	
aca Thr 65	cat His	ata Ile 1	aca g Thr Gl 70	lu Ly:	aa tat s Tyr <i>I</i>	gac ct Asp Lei 75	g tcc u Ser	Phe L	aag a eu Lys 80	gc atc Ser Ile	cag : Gln	240	
gaa Glu	gtt Val	Gly	ggc t Gly T 35	at gt yr Va	t ctt at al Leu 90	Ile Ala	gtc a Val A	at acg Asn Th 95	gtt tco r Val S	aaa a er Lys	tc :	288	
cct Pro	ctg (Leu	gag Glu 100	Asn I	tg cg Leu A	c atc a Arg Ile 105	att cgc Ile Arg	g Gly	cac to His Se 110	a ctt ta er Leu	t gaa g Tyr Glu	jac Asp	336	
aaa Lys	ttt (Phe 11	Ala	tg go Leu A	Ala V	ctg g al Leu 120	tg aac Val As	ttc a in Phe 12	e Asn	: agc at Asn Se	c gaa d r Ile Gl	caa u Gin	384	
Gly	gta Val 130	aaa Lys	gag t Glu L	ttg co eu Pr 135	o Leu	Thr Se	t tta : er Leu 140	act ga ı Thr (a ata c Glu Ile	tt aag g Leu Lys	ggt s Gly	432	
gga Gly 145	Val	aag Lys	Phe C	gc ag Cys A 50	g aac rg Asn	gat tat Asp T 155	yr Le	gt aat u Cys	gtg gg Asn Va 160	ig acc a il Giy T	atc hr Ile	480	
gag Glu	tgg Trp	Ala .	gac a Asp II .65	itc ct le Le	u Asn	atg aa Met Ly 70	g ago 's Ser	ctg c Leu F 175	Pro Thr	atc gtg Ile Val	agc Ser	528	
cat His	aat a Asn	ata a Ile 9 180	Ser Ty	it gga /r Giy	a aaa a y Lys A 185	aac tgt Asn Cy	s Gly	aag to Lys C 190	gc gat o ys Asp	cca ago Pro Se	tgt r Cys	576	
ttc a Phe	aat g Asn 19	Gly	cc tge Ser (Cys T	ggc a rp Gly 100	cc gga Thr G	ccc g ly Pro 20!	Asp l	g tgc c Lys Cys	ag aga s Gln Ai	atg rg Met	624	
Thr	aaa Lys 10	gtg Val 1	atc to lle Cy	gt go 's Ala 215	Glu G	iln Cys	t tca Ser 220	ggg ag Gly Ar	gg tgt a g Cys I	aaa gga Lys Gly	e ccc Pro	672	
aga Arg 225	Pro	att g Ile A	jac tg Asp Cy 23	ys Cy	aat g s Asn	aa cac Glu Hi 235	s Cys	ct gct Ala A	gga tg la Gly 240	ic act g Cys Thi	ga r Gly	720	
ccc Pro	aga Arg	Pro 1	aca ga Thr As 45	ac tgʻ sp Cy	ys Leu	cc tgt Ala Cy 50	aag g /s Lys	ac tto Asp I 255	Phe Glr	at gaa g n Asp G	ggg Ilu Gly	768	
aca	tgt a	aag g	зас д	ca tg	t ccg o	gg ctc	atg (ctc tac		ca aac a 93	aca ·	816	

	Lys Asp 260	o Ala Cys	265) Leu M	270	yr Asp Pro Asn	inr ·
	Leu Ala		Pro Tyr		s Tyr Se	c ttt ggg gca acc r Phe Gly Ala Th	
tgc atc a Cys Ile I 290	ag aca ys Thr	tgc cca Cys Pro 295	cac aac His Asn	tat gtg Tyr Va 300	gtg acg I Val Thr	gat cac ggg gcc Asp His Gly Ala	912
			Pro Gly		r Glu Va	ı gat gag ggt gg al Asp Glu Gly Gl 320	
						i aaa gtg tgc aat ro Lys Val Cys A	
						atc aat gcc acc er Ile Asn Ala Th	1056 ir
	Asp Ser		Asn Cy	s Thr L		ggc aat gtt gcc er Gly Asn Val Al	1104 a
						aac act tca gga r Asn Thr Ser Gl	1152 y
			ı Ser Va			aaa gaa atc act al Lys Glu Ile Th 400	1200 r
						cag tcc ctt agt et Gln Ser Leu S	1248 er
Ala Phe						a aaa aca caa gg r Lys Thr Gln Gl	
acg tac Thr Tyr 43	Ser Phe	gct gtc a Ala Val 44	Thr Lys	Thr Ala	atc act Ile Thr 15	cat tta ggc atg His Leu Gly Met	1344
						c atc gtt aag aat er Ile Val Lys Ası	
			r Ser Pro			a cgc ctc ttc aag ys Arg Leu Phe L 480	1440 ys
						gat gct gcc acc et Asp Ala Ala Th	
Cys Ala						c acg gct gac gg Cys Thr Ala Asp	

tgc tgg ggt ccc ggc ccc acc atg tgc ttc ggc tgt gag cat tac agc 1584 Cys Trp Gly Pro Gly Pro Thr Met Cys Phe Gly Cys Glu His Tyr Ser 515 520 525	1
cgc gga aaa cac tgc gtg gct tcc tgc aac ctg ctg aat ggt gag ccg Arg Gly Lys His Cys Val Ala Ser Cys Asn Leu Leu Asn Gly Glu Pro 530 535 540	32
cgt gaa tat gag gtc aat aaa aca tgc atg gaa tgc gat cct gaa tgt 1686 Arg Glu Tyr Glu Val Asn Lys Thr Cys Met Glu Cys Asp Pro Glu Cys 545 550 555 560	0
ctg ctc atg aat gaa acc cag acc tgc aac ggc cct gga ccc gac aaa 172 Leu Leu Met Asn Glu Thr Gln Thr Cys Asn Gly Pro Gly Pro Asp Lys 565 570 575	28
tgt aca gtg tgt gca aac tat aaa gac gga ccg cac tgt gtg cat cgc Cys Thr Val Cys Ala Asn Tyr Lys Asp Gly Pro His Cys Val His Arg 580 585 590	6
tgc ccg caa ggt gta cca gga gag aaa gac aca ctc atc tgg aaa tac Cys Pro Gin Gly Val Pro Gly Glu Lys Asp Thr Leu Ile Trp Lys Tyr 595 600 605	24
gct gac gtg aca cac gtt tgc cag ccc tgt cat gaa aac tgc acc cag Ala Asp Val Thr His Val Cys Gln Pro Cys His Glu Asn Cys Thr Gln 610 615 620	2
gga tgt acg ggg cct gat cta aag gac tgc aaa gat ttc aaa agc tct 192 Gly Cys Thr Gly Pro Asp Leu Lys Asp Cys Lys Asp Phe Lys Ser Ser 625 630 635 640	20
ggt ttg ccg atg atc gct ggc gtt gtc gga ggt cta ctg gcg ttt 1968 Gly Leu Pro Met Ile Ala Ala Gly Val Val Gly Gly Leu Leu Ala Phe 645 650 655	
gtt att ctg gct ctt gga gtg gcc gtt ctc ctg cgc aga cgc cac atc Val Ile Leu Ala Leu Gly Val Ala Val Leu Leu Arg Arg Arg His Ile 660 665 670	
cgg agg aag agg act ctg aga cga ctc ctg caa gag aga gag ctt gtg Arg Arg Lys Arg Thr Leu Arg Arg Leu Leu Gln Glu Arg Glu Leu Val 675 680 685	064
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<213> 斑马鱼([Danio rerio)		-

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- Leu Arg Met Tyr Arg Asn Cys Thr Val Val Leu Glu Asn Leu Glu Ile 50 55 60
- Thr His Ile Thr Glu Lys Tyr Asp Leu Ser Phe Leu Lys Ser Ile Gln 65 70 75 80
- Glu Val Gly Gly Tyr Val Leu Ile Ala Val Asn Thr Val Ser Lys Ile 85 90 95
- Pro Leu Glu Asn Leu Arg Ile Ile Arg Gly His Ser Leu Tyr Glu Asp 100 105 110
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- Gly Val Lys Phe Cys Arg Asn Asp Tyr Leu Cys Asn Val Gly Thr Ile 145 150 155 160
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- His Asn Ile Ser Tyr Gly Lys Asn Cys Gly Lys Cys Asp Pro Ser Cys 180 185 190
- Phe Asn Gly Ser Cys Trp Gly Thr Gly Pro Asp Lys Cys Gln Arg Met 195 200 205
- Thr Lys Val Ile Cys Ala Glu Gln Cys Ser Gly Arg Cys Lys Gly Pro 210 215 220
- Arg Pro Ile Asp Cys Cys Asn Glu His Cys Ala Ala Gly Cys Thr Gly 225 230 235 240
- Pro Arg Pro Thr Asp Cys Leu Ala Cys Lys Asp Phe Gln Asp Glu Gly 245 250 255

- Thr Cys Lys Asp Ala Cys Pro Arg Leu Met Leu Tyr Asp Pro Asn Thr. 260 265 270
- His Gin Leu Ala Pro Asn Pro Tyr Gly Lys Tyr Ser Phe Gly Ala Thr 275 280 285
- Cys Ile Lys Thr Cys Pro His Asn Tyr Val Val Thr Asp His Gly Ala 290 295 300
- Cys Val Arg Thr Cys Ser Pro Gly Thr Tyr Glu Val Asp Glu Gly Gly 305 310 315 320
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- Leu Asp Pro Ala Lys Leu Ser Val Leu Ser Thr Val Lys Glu Ile Thr 385 390 395 400
- Gly Tyr Leu Met Ile Gln Leu Trp Pro Glu Ser Met Gln Ser Leu Ser 405 410 415
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- Arg Glu Tyr Glu Val Asn Lys Thr Cys Met Glu Cys Asp Pro Glu Cys 545 550 560
- Leu Leu Met Asn Glu Thr Gln Thr Cys Asn Gly Pro Gly Pro Asp Lys 565 570 575
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- Ala Asp Val Thr His Val Cys Gln Pro Cys His Glu Asn Cys Thr Gln 610 615 620
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- Lys Ala Asn Lys Glu Ile Met Asp Glu Ala Tyr Val Met Ala Ser Val

760

- Glu His Pro His Val Cys Arg Leu Leu Gly Ile Cys Leu Thr Ser Thr 770 775 780
- Val Gln Leu Ile Thr Gln Leu Met Pro Tyr Gly Cys Leu Leu Asp Tyr 785 790 795 800
- Val Arg Glu Asn Lys Asp Arg Ile Gly Ser Gln His Leu Leu Asn Trp 805 810 815
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- Trp Ser Tyr Gly Val Thr Val Trp Glu Leu Met Thr Phe Gly Thr Lys 900 905 910
- Pro Tyr Asp Gly Ile Pro Ala Ser Glu Ile Ala Gly Val Leu Glu Lys 915 920 925
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- Val Asp Ala Asp Glu Tyr Leu Val Pro Asn His Ser Phe Phe Ser 1010 1015 1020
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INTERNATIONAL SEARCH REPORT

Inte onal application No.
PCT/CN03/01127

<u> </u>	1 Carron C. Land C. La		
A. CL	ASSIFICATION OF SUBJECT MATTER		
	IPC ⁷ A61K39/00; A61K39/12; (C12N15/00; C12N15/13, A61P35/00;	
According	to International Patent Classification(IPC) or to both n	ational classification and IPC	
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ļ	<u></u>	·	
B. FIE	LDS SEARCHED		
		·	
Minimum	documentation searched(classification system for	llowed by classification symbols)	
	IPC ⁷ A61K39/00; A61K39/12; (C12N15/00; C12N15/13, A61P35/00;	
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Documenta	ation searched other than minimum documentation to	the extent that such documents are included i	in the field searched
l			
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	1.1		
Electronic	data base consulted during the international search(n	ame of data base and, where practicable, se	arch terms used)
	CNPAT EPO	QUE(WPI), NCBI	
. .		QOE(WI I), NOBI	
C. DO	CHARACTE CONCIDENTA TO DE DEL EMAN		
	CUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where	appropriate, of the relevant passages	Relevant claim No.
A	WO, A2, 02092771, ((LUDW-N) LUD	WIG INST CANCER RES) 21	1-30
	NOVEMBER, 2002, see the abstract.	"TO INDI CHICER RES), 21,	1-30
Α	WO, A2, 0160317, ((REGC) UNIV CALIF	OPANA et al) 22 ATTCTTOT 2001	1.20
	see the abstract.	SKNIA, et al), 23, AUGUS1, 2001,	1-30
	see the abstract.		
		į.	4.00
Α	WO, A2, 0109303, ((VICA-N) VICAL IN	C), 08, FEBRUARY, 2001, see the	1-30
	abstract.		
Α	WO, A2, 0129242, ((MONS) MONSANT	O CO) 26 APPH 2001 1	1-30
, · ·	abstract.	0 CO),, 20, AFRIL, 2001, see the	
	, i		•
			•
Furtl	her documents are listed in the continuation of Box C.	See patent family annex.	
			<u> </u>
Special	categories of cited documents:	"T" later document published after the internal	tional filing date or priority
"A"document	defining the general state of the art which is not considered particular relevance	date and not in conflict with the applicati the principle or theory underlying the inve	on but cited to understand
	nument but published on or after the international filing date	"X"document of particular relevance; the claim	
	which may throw doubts on priority claim(s) or which is	considered novel or cannot be considered	to involve an inventive
cited to	establish the publication date of another citation or other	step when the document is taken alone	***
special	reason(as specified)	"Y"document of particular relevance; the claim considered to involve an inventive ste	ned invention cannot be
means	t referring to an oral disclosure, use, exhibition or other	combined with one or more other such doc	cuments, such combination
"P"document	published prior to the international filing date but later than	being obvious to a person skilled in the art "&"document member of the same patent family	
the prior	rity date claimed	ac document member of the same patent family	
Date of the	actual completion of the international search	Date of mailing of the international sea	1
	Total of the international search	Date of maning of the international sea	ich report
24 N	MAY 2004(24.05.2004)	16 TIMOOOM (10 - 0	6.2000
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NIomo	The Administration of		
INALLIE AND I	nailing address of the ISA/	Authorized officer	
	The Chinese Patent Office 6, Xitucheng Road, Haidian District,		
	Beijing, 100088, China	WEI Chunbaio	
Facsimile N	o. 86-10-62019451	Telephone No. 36-10-62085065	•
	SA/210(second sheet)(July 1992)	11_0 -0200000	

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna lapplication No.
PCT/CN03/01127

Patent document cited in search report	Publication date	Patent family members	Publication date
WO, A2, 02092771	21-11-2002	NONE	
WO, A2, 0160317	23-08-2001	US-A1-2002058041 AU-A-200136967 EP-A2-1255554	16-05-2002 27-08-2001 13-11-2002
WO, A2, 0109303	08-02-2001	NONE	·
WO, A2, 0129242	26-04-2001	AU-A-200115736 EP-A2-1224309	30-04-2001 24-07-2002

Form PCT/ISA/210(patent family annex)(July 1992)

PCT/CN03/01127

Α.	主题的	土公司

IPC⁷ A61K39/00; A61K39/12; C12N15/00; C12N15/13, A61P35/00; 按照国际专利分类表(IPC)或者同时按照国家分类和 IPC 两种分类

B. 检索领域

检索的最低限度文献(标明分类体系和分类号)

IPC7 A61K39/00; A61K39/12; C12N15/00; C12N15/13, A61P35/00;

包含在检索领域中的除最低限度文献以外的检索文献

在国际检索时查阅的电子数据库(数据库的名称和,如果实际可行的,使用的检索词)

EPOQUE(WPI), CNPAT, NCBI

C. 相关文件

ı	类 型*			
I	! 	引用文件,必要时,包括相关段落的说明	相关的权利要求编号	
	A	WO, A2, 02092771, ((LUDW-N) LUDWIG INST CANCER RES), 21, 11 月, 2002, 见摘要.	1-30	
	. A	WO, A2, 0160317, ((REGC) UNIV CALIFORNIA 等), 23, 8 月, 2001, 见摘要.	1-30	
	Α	WO, A2, 0109303, ((VICA-N) VICAL INC), 08, 2 月,2001, 见摘要.	1-30	
	A	WO, A2, 0129242, ((MONS) MONSANTO CO), 26, 4 月, 2001, 见摘要.	1-30	

■ 其余文件在 C 栏的续页中列出。

见同族专利附件。

- * 引用文件的专用类型:
- "A"明确表示了一般现有技术、不认为是特别相关的文件
- "E" 在先文件,但是在国际申请日的同一日或之后公布的 "L" 对优先权要求可能产生怀疑或者用来确定另一篇引用文
- "O" 涉及口头公开、使用、展览或其他手段的文件
- "P" 在国际申请日之前但迟于所要求的优先权日公布的文件

"T"在国际申请日或优先权日之后公布的在后文件,它与申请不 相抵触,但是引用它是为了理解构成发明基础的理论或原理

"X"特别相关的文件,当该文件被单独使用时,要求保护的发 明不能认为是新颖的或不能认为具有创造性

"Y"特别相关的文件,当该文件与其他一篇或多篇这类文件结合在一起,这种结合对本领域技术人员是显而易见的,要求保护的发明不能认为具有创造性

"&"同族专利成员的文件

国际检索实际完成的日期

245月 2004(24.05.2004)

国际检索报告邮寄日期 10・6月2004(10・06・2004)

国际检索单位名称和邮寄地址

中国专利局

中国北京市海淀区西土城路 6 号(100088)

传真号:

86-10-62019451

授权官员

魏和彭观

电话号码:

6,10,6208506

PCT/ISA/210 表(第 2 页)(1992 年 7 月)

国际检索报告 同族专利成员的情报

国际申请号

PCT/CN03/01127

PCT/CN03/0				
检索报告中引用的 专利文件	公布日期	同族专利	则成员	公布日期
WO, A2, 02092771	21-11-2002	无		
WO, A2, 0160317	23-08-2001	US-A1-200 AU-A-200 EP-A2-12	136967	16-05-2002 27-08-2001 13-11-2002
WO, A2, 0109303	08-02-2001	无		
WO, A2, 0129242	26-04-2001	AU-A-200 EP-A2-12		30-04-2001 24-07-2002
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			·	·
				·
DCT/ICA/210 主/日本土和四			·	

PCT/ISA/210表(同族专利附件)(1992年7月)